Ref. No. _____

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

SEROPREVALENCE, RISK FACTORS ANALYSIS AND OUTBREAK INVESTIGATION OF FOOT AND MOUTH DISEASE IN SELECTED AREAS OF CENTRAL AND SOUTHERN PARTS OF ETHIOPIA

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JUNE, 2025

BISHOFTU, ETHIOPIA

FOOT AND MOUTH DISEASE OUTBREAK INVESTIGATION IN SELECTED AREAS OF CENTRAL AND SOUTHERN PARTS OF ETHIOPIA

A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Microbiology

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JUNE, 2025

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Foot and Mouth Disease Outbreak Investigation in Selected Areas of Central and Southern Parts of Ethiopia

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STATEMENT OF THE AUTHOR

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ACKNOWLEDGEMENTS

First and above all, I want to express my gratitude to Almighty God for his help.

My heartfelt thanks go to my advisor, Dr. Eyob Hirpa, for his intellectual support, insightful advice, generous allocation of time, and meticulous corrections throughout the process of this MSc thesis.

I am deeply grateful to the Director General, Dr. Tesfaye Rufael, for his invaluable guidance and support. His assistance with financial matters and in pursuing my MSc program has turned my dream into a reality, thanks to his consistent follow-up. I would also like to express my gratitude to all the staff at AHI, particularly Dr. Daniel Gizaw, Mrs. Ayelech Muluneh, Mrs. Tsion Blatta, Dr. Garoma Desa, Mr. Tekele Warku, Mr. Damasa Negessu, and Mr. Kamal Emiyu, for their exceptional support during the sample collection, laboratory processing, and testing phases. Furthermore, I would like to thank my team at the Kality tsetse fly research center.

Additionally, I would like to thank all the CVMA staff for their assistance in the dormitory, library, and 24-hour internet service during my MSc thesis write-up.

My special thanks also go to my family, my wife Birane Basha, and my children, first son Bilisa, Ketoran, and new baby Bethlehem, for their patience in living alone in my absence and her devotion to taking care of the family in every aspect.

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LIST OF ABBREVIATIONS

3ABC-ELISA	3abc-Enzyme Linked Immune Sorbent Assay
AHI	Animal Health Institute
BHK21	Baby Hamster Kidney 21
BSL2	Biosafety level two
Cdna	Complement De-Oxy Ribonucleic Acid
CPE	Cytopathic effect
DIVA	Differentiating Infected From Vaccinated Animal
DNTPs	Deoxy-Nucleotide Triphosphate
ELISA	Enzyme Linked Immune Sorbent Assay
FMD	Foot and Mouth Disease
FMDV	Foot And Mouth Disease Virus
GDP	Gross Domestic Product
IRES	Internal Ribosomal Entry Site
KAP	Knowledge Attitude and Practices
MAbs	Monoclonal Antibody
MHC	Major Histocompatibility complex
NSP	Non-Structural Protein
OIE	Office Of International Epizootics
ORF	Open Reading Frame
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RPSA	Ribosomal Protein SA
RRT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
RT-LAMP	Reverse Transcription Loop Mediated Isothermal Amplification
SAT	South African Territories
SPC-ELISA	Solid phase Competitive ELISA
UTR	Untranslated Region
WRLFMD	World Reference Laboratory Foot And Mouth Disease

ABSTRACT

Foot and mouth disease is a highly contagious Trans boundary disease causing significant economic losses in cattle in Ethiopia that caused by Virus belonging to the family *Picornaviridae's* genus *Aphthovirus.* A cross sectional study and outbreak investigation was performed from October 2023 to May 2025. The study included selected areas five regions of Ethiopia. The study objectives were to estimate the seroprevalence of FMD, identifying circulating serotypes in the study areas. The seroprevalence was determined by 3ABC-ELISA and the circulating serotype obtained from antibody was identified by SPC-ELISA. In active Outbreak area the virus was cultivated on BHK 21 Cell line and virus genome detected using RTPCR. The circulating serotype identified by antigen captured ELISA (IZSLER, Brescia, Italy). Semi-structured questionnaire survey was used to assess the community's knowledge's, attitude and practice toward FMD. The overall seroprevalence from 314 cattle was 80.89% (254/314). The serotyping result from 22 strong seropositive selected samples showed concurrently 4 serotypes circulating in south Omo namely, the SAT2 (18/22), O (16/22), SAT1 (13/22) and A (10/22). There was statistically significant p-value difference in seroprevalence in South Omo Zone districts. Totally 28 sample were cultivated on BHK21 cell lines took from 54 animals of outbreak investigation area for virus isolation was shown 64 %(18/28) samples of *FMDV* CPE positive and whereas, 28 sample pooled into 22 sample by locality for *FMDV* genome detection by RT-PCR. The molecular detection confirms 45% positive for *FMDV*. For serotyping 18 samples positives for *FMDV* CPE were used and two circulating serotypes were identified namely, serotype O, 66.7% (12/18), and 16.7% (3/18) of serotypes A, and mixed serotypes O and A, 16.7% (3/18). Knowledge, Attitude and Practices survey analyses showed the 95% respondent do not vaccinate their animal for FMD and do not quarantine diseased animal. In conclusion FMD is very important economical diseases in the study area therefore, awareness creation and Vaccination campaign to protect the Cattle against FMD is recommended.

Key words: *Foot and Mouth disease, Isolation, Knowledge, Attitude and Practices, Molecular Detection Outbreak Investigation, Sero-prevalence.*

1. INTRODUCTION

Ethiopia possesses Africa's largest livestock population, with over 65 million cattle ranking fifth globally (CSA, 2020). This sector underpins livelihoods for 80% of the rural population and contributes 45% to agricultural GDP (World Bank, 2017). Export of live animals, meat, hides, and skins generates critical foreign exchange, accounting for 16-19% of national GDP (Abdela, 2017; Wakaso *et al.,* 2025). Despite this economic centrality, cattle productivity remains suboptimal due to multifaceted constraints including endemic diseases, recurrent droughts, climate variability, inadequate veterinary services, and poor nutrition (Habte *et al.,* 2022). Among diseases, Foot and Mouth Disease (FMD) represent a primary barrier to productivity and trade. Foot and Mouth Disease is a highly contagious transboundary disease affecting cloven-hoofed animals including cattle, sheep, goats, pigs, and wildlife (OIE, 2009).

The causative agent, *Foot and mouth disease virus (FMDV)* belongs to the genus *Aphthovirus* within the *Picornaviridae* family. This non-enveloped, single-stranded RNA virus was first isolated by Loeffler and Frosch in 1897. *FMDV* exhibits high genetic diversity comprising seven immunologically distinct serotypes: O, A, C, Asia 1, SAT1, SAT2, and SAT3 (Grubman & Baxt, 2004). Global distribution of these serotypes shows significant heterogeneity. Serotypes O and A demonstrate pandemic distribution circulating widely across Africa, Asia, and South America. The SAT serotypes (SAT1-SAT3) remain largely restricted to sub-Saharan Africa, while Asia 1 predominates in South Asia. Notably, serotype C is now considered extinct in wild populations. Major FMD-free regions include Europe, North and Central America, Australia, and New Zealand (Knowles & Samuel, 2003; OIE, 2017).

The disease inflicts severe economic losses through multiple pathways. Direct production impacts include reduced milk yield (up to 80%), significant weight loss, abortion rates, and high mortality in young stock due to myocarditis (MacLachlan and Dubovi, 2011). International trade restrictions impose secondary economic damage through bans on livestock and livestock products from endemic regions (OIE, 2018). Control costs further burden economies through vaccination programs, surveillance

systems, and outbreak containment measures (Wubshet *et al.,* 2019). In Ethiopia specifically, FMD affects approximately 77% of cattle in the Horn of Africa region, with estimated annual losses reaching USD 1.5 billion through combined productivity deficits and missed trade opportunities (Abdela, 2017; Woldemariyam *et al.,* 2023).

Foot and Mouth Disease maintain endemic status across sub-Saharan Africa with complex serotype distributions. In Ethiopia, Kenya, Sudan and South Sudan, serotypes O, A, SAT1 and SAT2 circulate commonly. Uganda primarily experiences SAT3 outbreaks, while Eritrea reports serotypes O, A and SAT2 (Compston *et al.,* 2021; Woldemariyam *et al.,* 2023). Recent outbreaks involving SAT2 and SAT1 serotypes have been documented across numerous African nations including Algeria, Botswana, the Democratic Republic of the Congo, and South Africa, with SAT1 topotype III specifically identified in Botswana (WRLFMD, 2013, 2018). Several factors facilitate transmission in the region: pastoral mobility enables seasonal cattle movements across international borders; limited biosecurity measures result in fewer than 10% of farms implementing quarantine protocols (Aman *et al.,* 2020); and vaccine mismatch issues occur due to poor serotype coverage in commercially available vaccines (Tesfaye *et al.,* 2020).

Ethiopia has documented FMD as endemic since its first official report in 1957 (Martel, 1974). Hyperendemic zones with particularly high prevalence include pastoral systems such as Borana (seroprevalence 23.4-41.8%), South Omo (32.6-68.2%), and Afar (27.3%) (Molla *et al.,* 2010; Gizaw *et al.,* 2025). Highland regions including Arsi experience annual outbreak incidence rates of 17.8%, with significant disease pressure also documented in Gamo zone (Tolawak *et al.,* 2023). Western and northern regions such as Benishangul-Gumuz and Amhara similarly report persistent outbreaks (Muche *et al.,* 2021; Shurbe *et al.,* 2022). Despite this well-established endemic status, critical knowledge gaps impede effective control. Serotype dynamics remain poorly understood due to insufficient concurrent surveillance across outbreak and non-outbreak areas. Diagnostic limitations persist through overreliance on serology without complementary virus isolation during outbreak investigations. Control programs face challenges with national vaccination coverage remaining below 15% (Dubei & Negash, 2021). Policy development is constrained by inadequate epidemiological data required for establishing FMD-free zones.

This study addresses these evidence gaps especially the FMD transmission way in pastoral areas and the molecular data shortages in the study area through implementation of integrated diagnostics combining serology, virus isolation, and real time RT-PCR in selected districts of five regions of Ethiopia. By simultaneously investigating areas with active outbreaks and regions without reported cases, this research generates organized data on serotype distribution and transmission routes. Findings will directly inform evidence-based control strategies aligned with Ethiopia's National Livestock Master Plan (2021-2030), ultimately supporting the country's objectives to secure international market access for livestock products through improved disease management.

1.1 Problem Statement

Ethiopia's strategic initiative to expand live animal exports to China, Europe, and Asia faces significant obstruction due to endemic Foot and Mouth Disease. Persistent outbreaks occurring even in vaccinated herds systematically undermine international market access by contravening international requirements for trade. While the Ethiopian government prioritizes establishing FMD-free area to enable exports, this plan remains unattainable without resolving critical evidence gaps. Existing epidemiological studies on FMD in Ethiopia demonstrate uncoverable molecular data and methodological limitations, with most research narrowly focused on seroprevalence assessments. These investigations fail to integrate comparative analysis of active outbreak zones versus disease-no out break free regions, lack comprehensive serotype characterization across epidemiological contexts, and omit combined serological-virological molecular diagnostics. Consequently, current data inadequately inform control strategies by neglecting transmission dynamics and serotype-specific risks in diverse settings. This study addresses these deficiencies through implementation of integrated diagnostics across outbreak and non-outbreak areas in five Ethiopian regions. By simultaneously investigating seroprevalence rates and active virus circulation, identify circulating serotypes in both scenarios and contribute for establishing the essential foundation for targeted vaccination programs, effective surveillance systems, and evidence-based policies for FMD-free zone development.

General Objective

To generate organized evidence for Foot and Mouth Disease (FMD) control in Ethiopia through integrated assessment of seroprevalence, circulating serotypes, outbreak, and community KAP Assessment.

Specific Objectives

- To estimate seroprevalence and identify circulating serotypes of FMD in South Omo Zone using 3ABC-ELISA and SPC-ELISA.
- To identify risk factors for FMD prevalence in the study area.
- To characterize *Foot and mouth disease virus (FMDV)* in active outbreak areas through virus isolation using BHK-21 cell culture, molecular confirmation via real time RT-PCR, and serotype identification by antigen-capture ELISA.
- To assess community KAP regarding FMD transmission, prevention, and reporting through semi-structured questionnaires.

2. LITERATURE REVIEW

2.1 Etiological agent of *FMDV*.

Foot and mouth disease is a contagious viral infection caused by member of the *Picornaviridae* of genus *Aphthovirus*. It is a highly contagious disease of cloven hoofed ungulates characterized by fever and the formation of vesicles on epithelial surfaces. FMD is a known List **A** disease that has major economic importance internationally because of its rapid invasions and the major economic losses causes in susceptible animals (Park *et al.,* 2022; Quinn *et al*., 2011).

The virus has tiny single stranded RNA viruses which have no envelope and it organizes the viral proteins VP0, VP1, and VP3 into twelve pentamers, each of which has 60 copies, and forms an icosahedral capsid. All three of the Viral proteins (VP1,VP2 and VP3) are surface exposed and adopt an 8 β-barrel-type stranded form with extended N and C termini, with VP1 encircling the 5 fold axes of symmetry and VP2 and VP3 alternatively enveloping the icosahedral 3-fold axis (Park *et al.,* 2022; Wang and Liu, 2020).

Viral protein 1 (VP1) is the most important structural protein of *FMDV*, controls the host's immune response and serotype. VP1 exists in the capsid, a portion of the virus's outer layer that protects its genetic material and interacts with host cell receptors. VP1 is the most exposed and changeable protein on the capsid surface. It contains the primary *FMDV* antigenic site, which is recognized by the host's antibodies. Furthermore, VP1 contributes to *FMDV's* binding to two host cell receptors, integrins and heparan sulfate proteoglycans. VP1 can change its antigenicity and structure to avoid the host's immune response and infect new cells (Malik *et al.,* 2017; Domingo *et al*., 2005; Knowles *et al*., 2003).

2.1.1 The foot and mouth disease virus genome

The *Foot and mouth disease virus* is a positive sense single stranded RNA virus with a roughly 8.5 kb genome. The genomic RNA is enclosed by a capsid *FMDV* virion. The genome RNA is encodes a single long open reading frame (ORF) with two different start sites that is about 7 kb long. The ORF of *FMDV* genome is flanked by two untraslated region (UTR) called a 5′ UTR and a 3′ UTR. The 5′ UTR ends with the viral coding peptide VPg, which is covalently joined to the genome. The internal ribosome entry site (IRES), poly(C), pseudo knot, cre structures, S fragment (a short portion of the genome), and poly(C) are all present in the *FMDV's* 5′ UTR. The homopolymeric cytidylic acid tract (poly(C)), which isolates the S segment from the genome and allows it to form a stem-ring structure with around 350 bases, varies in length depending on the serotype. The S fragment can prevent daughter RNA from being damaged by nucleic acid exonuclease and can significantly increases the viral RNA to be replicate successfully (Wang and Liu, 2020; Grubman and Baxt, 2004).

Foot and mouth disease virus RNA 3′-UTR region consists of two components, a structural sequence of 90 nucleotides folding into two separate stem-loops and a poly (A) tail with variable length. Both virulence and viral replication are modulated by these two factors. The structured 3′-UTR uses a particular long range RNA–RNA interaction pattern to directly attach to S-fragment and IRES elements at two different locations in the 5′-UTR. The IRES-3′-UTR interaction, which is independent of the poly (A) tail and increases IRES activity, requires both of the 3′-UTR stem-loop structures. The S-fragment, however, depends on the poly (A) tail and interacts with every stem-loop. These results showed that the 3′-UTR controls the pathogenicity of *FMDV* and increases IRES activity (Gao *et al.,* 2016; Serrano *et al.,* 2006; Belsham, 1993).

On the other hand, Foot and mouth disease virus have more 10 nonstructural protein (NSP) namely 3B, L, 2A, 2B, 2C, 3A, 3B1, 3B2, 3C, and 3D and four structural proteins (VP1, VP2, VP3, and VP4) (Wang and Liu, 2020; Gao *et al.,* 2016). However, due to the absence of a proofreading mechanism in the RNA polymerase expressed by the *FMDV*, the virus genome shows a very rapid mutation rate (Rai *et al.*, 2017). The rapid evolution of *FMDV*, which has given rise to its high mutation

rate, quick population growth, and seven primary serotypes (A, O, C, Asia1, South African Territories (SAT) 1, SAT2, and SAT3), is a result of the virus's rapid evolution. Each serotype has also given rise to a large number of variations and subtypes (Park *et al.*, 2022; Wang and Liu, 2020; Liu *et al.*, 2018).

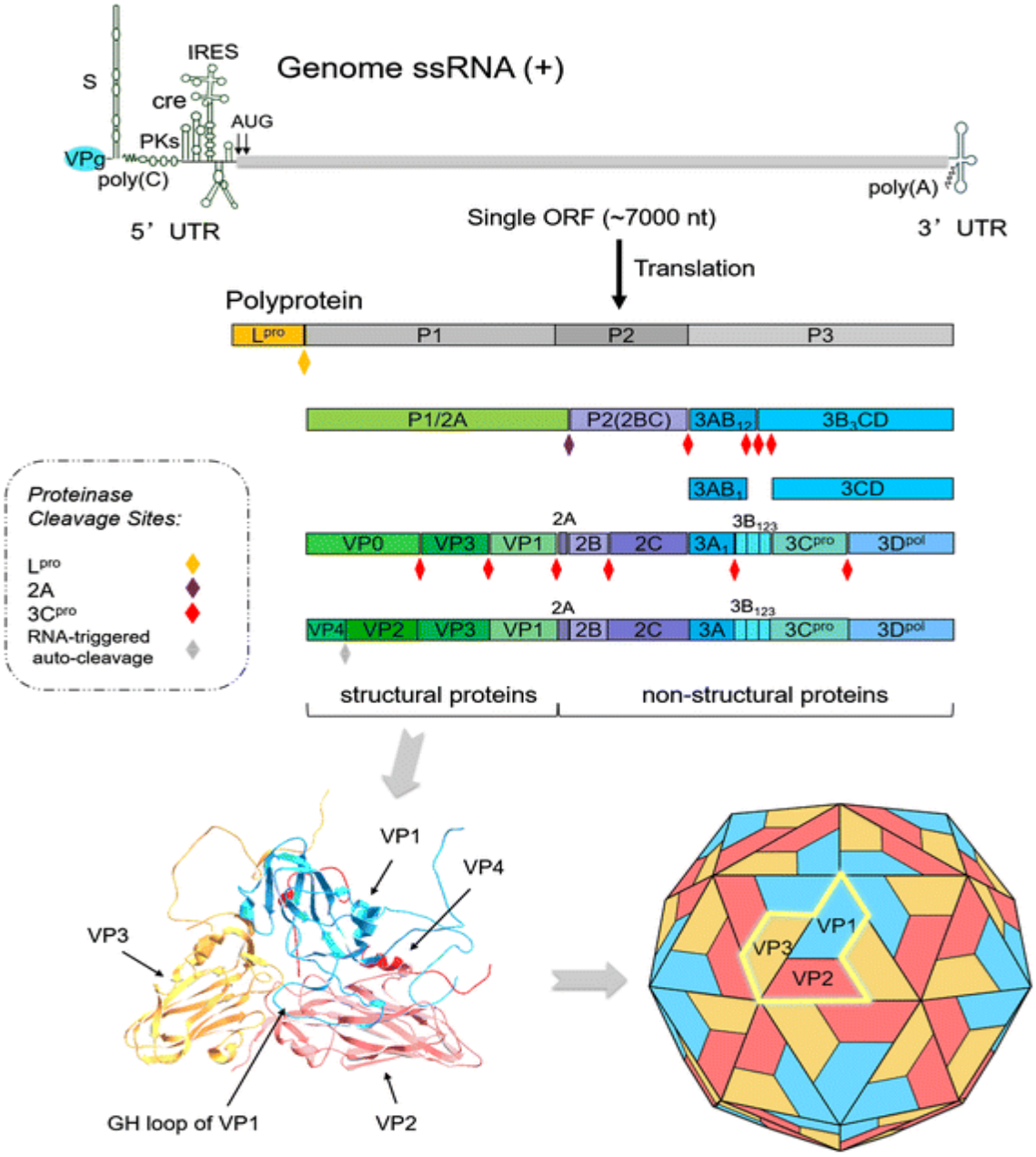


Figure 1: Diagram illustrating the *FMDV* genome, viral polypeptids and structural protein conformations. (Gao *et al.*, 2016).

2.1.2 Structural proteins

Foot and mouth disease virus is made up of the four capsid proteins namely VP1, VP2, VP3, and VP4, of which VP1, VP2, and VP3 are found in the virus's outermost layer and VP4 is found inside the virus and makes contact with RNA. Likewise, *FMDV* structural protein (VP1) may play a role in the initial interactions between the virus and host cells. *FMDV* VP1 binds to RPSA (ribosomal protein SA) and inhibits the RPSA-mediated regulatory effect on host MAPK pathway (Mitogen Activated Protein Kinase) activation. The researchers discovered that the point mutations of the VP1 were the cause of the mouse virulence and BHK21 cell pathogenicity (Zhu *et al.*, 2020; Malik *et al.*, 2017; Dawe and King, 1983).

The VP1 protein can cause the body to develop certain neutralizing antibodies and establish anti infection immunity because the majority of the VP1 protein is exposed on the surface of the virus, which greatly influences the virus's antigenicity (Burma *et al.,* 2006). This feature of VP1 was exploited by researchers to conduct numerous investigations in the creation of *FMDV* vaccine. Additionally, research has demonstrated a connection between the pH stability of *FMDV* particles and the VP1 N terminus has playing important role in modulating of the *FMDV* virions stability in acidic environment (Zhu *et. al.*, 2020; Caridi *et al.*, 2015).

The structural protein VP2 is the viral protein contributes to *FMDV* persistence in the host cells and VP3 can enhance the acid resistance of serotype A. Furthermore, the researchers found that FMDV VP3 inhibited the IFN-beta signaling pathways and the IFN-gamma signal transduction pathways. However, *FMDV* VP4 may be involved in inducing an immune response in T cells to recognize the T cell epitopes of MHC, a property that could be used to develop peptide vaccines (Li *et. al.,* 2016; Vazquez-Calvo *et al.,* 2014; Blanco *et. al.,* 2000).

2.1.3 Nonstructural proteins of the FMDV

The *Foot and mouth disease virus* NSPs involved in virus RNA replication and protein processing are produced from the P2 and P3 precursors (Belsham, 1993). The *FMDV* ORF has two initiation codes called AUG, which when used to make lead proteases can result in the production of two different types, Lab and Lb. however, the three mature peptides, 2A, 2B, and 2C, are finally produced from the P2 component of *FMDV*. The *FMDV* 2A protein has the ability to cleave the 2A/2B site, because the 2A polypeptide possesses the conserved c-terminal pattern, where the final P is the first residue of 2B, and it is critical for protein processing and virus replication. It has been demonstrated that the 2A polypeptide can cleave the 2A/2B junction (Wang and Liu, 2020).

Non-structural protein 3A, which was more specific and sensitive than other non-structural proteins 3B and 3AB, was also used by the researchers to identify infected and immunized mice. The 3B protein, also known as VPg, is present in *FMDV*'s genome three times. In order to avoid the host immune system, 3A was also discovered to block interferon beta signaling. The 3C protease split the *FMDV* capsid precursor, P2-2A, into VPO, VP3, VP1, and 2A, and these three cleavages occurred independently of one another. The final non-structural protein is 3D polymerase, an RNA-dependent RNA polymerase and microRNAs that specifically target 3D polymerase can successfully stop *FMDV* proliferation in vitro. As a result, one of the efficient targets for the creation of antiviral medications that specifically target *FMDV* is 3D polymerase. The *FMDV* replication in host cells can be successfully stopped by the 3D polymerase inhibitor 5D9 (Wang and Liu, 2020; Gao *et al.*, 2016).

2.1.4 Serotypes of the FMDV

The *Foot and mouth disease virus* has seven different serotypes, labeled A, O, C, Asia 1, SAT 1, SAT 2, and SAT 3, and each serotype has numerous subtypes. Moreover, within these serotypes, over 65 diversities of topotypes, genetic lineages, and strains have also been identified using biochemical and immunological tests. FMD outbreaks have been reported throughout the majority of the world that the serotype O is the most prevalent. Six of the seven serotypes; A, O, C, SAT1, SAT2, and SAT3 have

been found in Africa, compared to four in Asia (O, A, C, Asia1) and just three in South America (O, A, C) (Wang and Liu, 2020; Lewis-Rogers *et al.*, 2008).

From the year 1957 to 2004, the serotype C virus was prevalent in East Africa including the most recently recovered Kenyan isolate from 2004 and any cases of serotype C hasn't confirmed in over 15 years from the outside of laboratory (Paton *et al.*, 2021). *FMDV* is also characterized by its high mutation rate, which ranges from 10^{-3} to 10^{-5} mutations per nucleotide site per replication due to the lack of RNA polymerase proof reading ability in a quasi-species structure of *FMDV* population. Genetic variation in the capsid encoding region may change viral epitopes and result in the formation of novel antigenic variants, compromising the efficacy of vaccine (Abdulrahman *et al.*, 2019).

2.2. Epidemiological description of FMD

The epidemiology of FMD depends on the genomic and antigenic investigations of *FMDV* that it exists in various parts of the world and is categorized into seven regional *FMDV* pools. The distribution of *FMDV* is also characterized by 7 geographical endemic pools with different viral lineages. Likewise, the Pool 1 was circulated in East Asia, Pool 2 in South Asia, Pool 3 in West Eurasia, Pool 4 in East Africa, Pool 5 in West Africa, Pool 6 in Southern Africa and Pool 7 in South America (Al-Rawahi *et al.*, 2024).

Accordingly, there are three continental epidemiological clusters for FMD worldwide, the South America, Asia, and Africa. The distribution of the virus worldwide seems to align with patterns of animal movement and trade, as well as specific wildlife reservoirs, such as African buffalo, within certain regions especially in African continent and the other is by an independent cycle that is maintained within domestic animals (Maree *et al.*, 2014; Jamal and Belsham, 2013).

The global distribution of FMD was depending on the seven virus pools that make up the disease to be existed. Six (A, O, C, SAT1, SAT2, and SAT3), four (A, O, Asia1, C), and three (A, O, C) serotypes have historically been found in Africa, Asia, and South America, respectively. FMD is endemic in the Horn of Africa, with different

countries having different prevalence rates (Woldemariyam *et al.,* 2023). The main way that the disease is spread is by inhaling virus particles through direct contact with the breath of animals that are acutely infected. Cattle with aerosolized virus infections typically transmit the infection through their respiratory systems and Infection can also result from skin or mucous membrane abrasions (Grubman and Baxt, 2004).

The Ideal conditions for virus survival are temperatures below 50 °C, relative humidity above 55% and neutral PH. Airborne transmission has also been implicated in the spread of disease over both long (up to 50 km over land and 200 km over water) and short distances (within 2 km proximity) (Brown *et al.,* 2022). Moreover, some factors that affect the risk of airborne FMD transmission are the virus strain or serotype, topographic factors, animal type and number infected (i.e., virus production and concentration), and weather conditions that influencing viral decay (Brown *et al.*, 2022; Hagerman *et al.,* 2018).

Foot and mouth disease primarily affects animals with cloven hooves and Cattle are the primary hosts, while certain FMDV strains can adapt to pigs. Wild pigs, antelopes, and deer also harbor *FMDV* strains that infect cattle, making them significant hosts for natural infection. There is little to no epidemiological significance to the infections that occur incidentally or accidentally in other susceptible species, such as Giraffes, deer, antelope, wild pigs, and elephants. Although the exact mechanism of resistance is unknown that horses are resistant to FMD. Food safety and human health are unaffected by FMD, but only healthy animals should be consumed. However, because FMD lowers animal productivity and income, it can completely destroy impoverished communities' livelihoods and food security (Zewdie *et al.,* 2023; Grubman and Baxt, 2004).

2.3 Clinical signs of the FMD

The FMD symptoms vary based on strain, exposure, age, species, and host immunity. Morbidity can reach 100% in sensitive populations. Incubation takes 2-14 days. Symptoms include lameness, blisters, fever, depression, hyper salivation, weight loss, and decreased milk production, refusal to eat and walk or move compromising young animals' health. *FMDV* can occur in all fluids and excretions, including aerosols

infecting other animals. The respiratory tract is the main site for virus replication (Wong *et al.,* 2020; Grubman and Baxt, 2004; Davies, 2002).

2.4 Diagnostic methods

The *Foot and Mouth disease virus* can be detected using cell culture isolation, complement fixation testing, ELISA, PCR, diagnostic serology, and herd level separation of vaccinated and infected animals through antibody response to viral non-structural proteins (Remond *et al.,* 2002). The Virus Isolation (VI) was used to isolate the live virus from tissues of infected host animals, Sandwich ELISA (S-ELISA) used for serotyping of the isolated virus, Multiplex-PCR (m-PCR) for virus genome detection, and Indirect ELISA (DIVA) for identification of the viral nonstructural protein infecting the animals from structural protein of vaccinated animals (Longjman *et al.,* 2011).

ELISA is often divided into sandwich, competitive, indirect, and direct ELISA. In addition to virus isolation, VNT, and PCR-based methods, ELISA is currently one of the most widely used methods for FMDV detection. Abu Elzein and Crowther (Elzein and Crowther, 1978) were the first to report the use of an indirect ELISA to detect FMDV specific antibodies in vaccinated bovine sera. The test sera from cattle reacted with the FMDV coated on the microtiter plate, and the anti-bovine antiserum conjugated to an enzyme was then used for detection. It was reported that the presence of procomplementary or anticomplementary factors in the samples, as well as the 12S antigen, had no effect on the sandwich ELISA's ability to identify FMDV specifically, in contrast to CFT. Additionally, because fewer sera are needed for each test, sandwich ELISA is typically less expensive than CFT and enables immediate evaluation of material without virus isolation. Furthermore, the heterogeneity in tissue culture susceptibility has little effect on ELISA. ELISA was also shown to be more reproducible than VNT in comparative investigations using repeated testing of sera (Wong *et al.,* 2020).

2.5 Prevention and Control

The prevention of FMD has been greatly aided by vaccination (Sutmoller *et al.,* 2000). Vaccination campaigns are conducted in FMD endemic areas as a preventive

control measure or to reduce disease effects, using adjuvants like oil emulsions and chemically inactivated particles. Large volumes of live virus have to be grown in order to produce inactivated vaccines, which necessitates facilities with high biosecurity standards and increases the risk of virus escape, which might hinder vaccine production in areas free of foot and mouth disease (Capozzo *et al.,* 2023).

Preventive measures may be implemented in three main ways to decrease the chance that FMD will infect and spread throughout the farm. The first is by putting strict biosecurity regulations in place for people, cars, equipment, animals, and animal derived goods. Moreover, to prevent the disease from spreading restricting all animal movement is the good practices of controlling the disease (Yano *et al.,* 2018).

2.6 Economic Importance

Although many wealthier countries have successfully eradicated FMD from their herds of animals in their country, despite the disease is still widespread in most of the world. The economic impact of FMD outbreaks is evident when they happen in disease free countries and areas where livestock is raised for export. Among the visible losses happened due to FMD both adults and calves directly were affected and is responsible for 33% of losses in endemic areas when it comes to milk production. Long term FMD slows animal growth rates, raises death rates, and reduces milk output by 80%. This short term immunity limits resources for other diseases. Additionally, it might result in abortions, a decrease in traction power, especially during harvest, and expensive expenses for farmers to maintain the cow without production (Knight Jones and Rushto, 2013). However, The FMD's economic impact in endemic regions is estimated to be between 6.5 and 21 billion USD annually (Chanchaidechachai *et al.*, 2022).

Pigs in intensive production systems and dairy cattle which are the crucial sources of animal protein in poor countries are experiencing visible production losses. Fertility problems due to abortion and reduced conception rates lead to a need for more breeding animals, resulting in an invisible loss in meat and milk production (Chaters *et al.,* 2018). Wildlife is often restricted from FMD free zones through costly fencing, affecting wildlife ecology like African Buffalo. Despite being FMD free, ongoing

costs include preventing disease introduction, maintaining early post mortem detection and control capabilities, research, and livestock restrictions on the livestock sectors are still other problems of economic losses in order to manage the disease not to enter the disease free zone areas (Knight Jones and Rushto, 2013; Sutmoller *et al.*, 2000).

Ethiopia has the largest cattle population in Africa, due to this High production costs and challenges in meeting export standards limit market access for meat exporting nations, leading to increased livestock purchases through unofficial channels at lower prices. High production costs and challenges in meeting export standards limit market access for meat exporting nations, leading to increased livestock purchases through unofficial channels at lower prices. Likewise, FMD is the main viral disease causing significant economic losses in Ethiopia that losses due to FMD cases per year was estimated 0.9 million US$ per year (Rasmussen *et al.,* 2024; Dubei and Amare, 2020; Knight Jones and Rushto, 2013).

2.7. Status of Foot and mouth disease in Ethiopia

Ethiopia has been reporting cases of foot-and-mouth disease (FMD), a disease that requires notification, since 1957 (Martel, 1974). To this day, FMD remains one of the top five economically significant endemic viral diseases affecting cattle in Ethiopia (Waldemariyam *et al.,* 2023). Moreover, Ethiopia is home to four endemic serotypes, the serotype O, A, SAT 1, and SAT 2. Serotype O is the most prevalent and widespread. The effective control and prevention measures in Ethiopia are limited due incomplete understanding of epidemiology and evolution of the *FMDV* strains circulated in Ethiopia. Outbreaks of FMD are common throughout the country, reaching its highest in frequency and severity during the dry season. Numerous factors, including the production system, geographic location, species, age of the animals, contact with wildlife, season of the year, mixed animal species, breed, and agro ecology, affect the frequency and distribution of FMD outbreaks (Zewdie *et. al.,* 2023).

According to the seroprevalence study conducted so far in this country, the prevalence in cattle ranged from 4.8% to 72.1%. However, there are regional differences in the

disease's prevalence. Five *FMDV* serotypes (O, A, C, SAT1, and SAT2) are currently identified and documented in Ethiopia. The serotype O was highly prevalent and dominant serotype causing most of the FMD outbreaks in Ethiopia (Gelana *et al.,* 2016; Awel *et al.,* 2021).

Table 1: Summary report Foot and Mouth disease in Ethiopia.

No.	Region/ Zone	Techniques	prevalence	Serotype	Authors
1	Amhara	3ABC ELISA	14.9%		Tesfaye *et. al.,*, 2016
2	Oromia	3ABC ELISA	15.0%		Hordofa *et. al.,*, 2018
3	Central Ethiopia	Molecular	NA	O, A and SAT2	Sulayeman *et. al.*, 2018
4	Amhara	3ABC ELISA	11.48%		Mesfine *et. al.,*, 2019
5	Oromia	3ABC ELISA	40.4%		Ahmed *et. al.,*, 2020
6	Northern & Central Ethiopia	Molecular	NA	A, O and mixed A and O	Tesfaye *et. al.,*, 2020
7	Afar	Molecular	NA	SAT2	Dubei and Amare, 2020
8	Oromia	Molecular	NA	O	Muchei *et. al.,*, 2021
9	Afar	3ABC ELISA	19.2%		Dubei and Negash, 2021
10	Oromia	Molecular	NA	O	Awel *et. al.*, 2021
11	Southern Ethiopia	3ABC ELISA	26.3 %		Shurbe *et. al.,*, 2022
12	Tigray	Molecular	NA	O and A	Tesfaye et. al.,, 2023
13	Oromia	3ABC ELISA	20.3%		Tolawak *et. al.,*, 2023
14	Addis Ababa	3ABC ELISA	56%	O and A	Seifu *et al.*, 2023
15	South Ethiopia	Molecular	NA	SAT2	Bandaw *et. al.,*, 2024
16	Oromia	Molecular	NA	A,O, SAT1, SAT2	Gizaw *et. al.,*, 2024
17	Oromia	Molecular	NA	A	Tegegn *et. al.,*, 2024
18	South-West Ethiopia	3ABC ELISA	5.3%		Reta, 2024

3. MATERIALS AND METHODS

3.1. Study area

The study encompassed five ecologically diverse regions in Ethiopia, characterized by distinct topographies and climatic conditions. The Sidama Region (Hawassa) features a tropical climate (15–19.9°C) with elevations of 1500–2500 meters and annual rainfall of 1200–1599 mm. Addis Ababa (Akaki Kality), the capital, lies at ~2300 meters, with a temperate climate (9.89–24.64°C) and high annual rainfall (~1874 mm). In the Oromia Region, Burayu/Shegar City's highland terrain (2712 m elevation) experiences cool temperatures (14.7°C mean), while Habas District (1918 m elevation) has a bimodal rainfall pattern (1500–2000 mm/year) and temperatures ranging from 12–28°C.

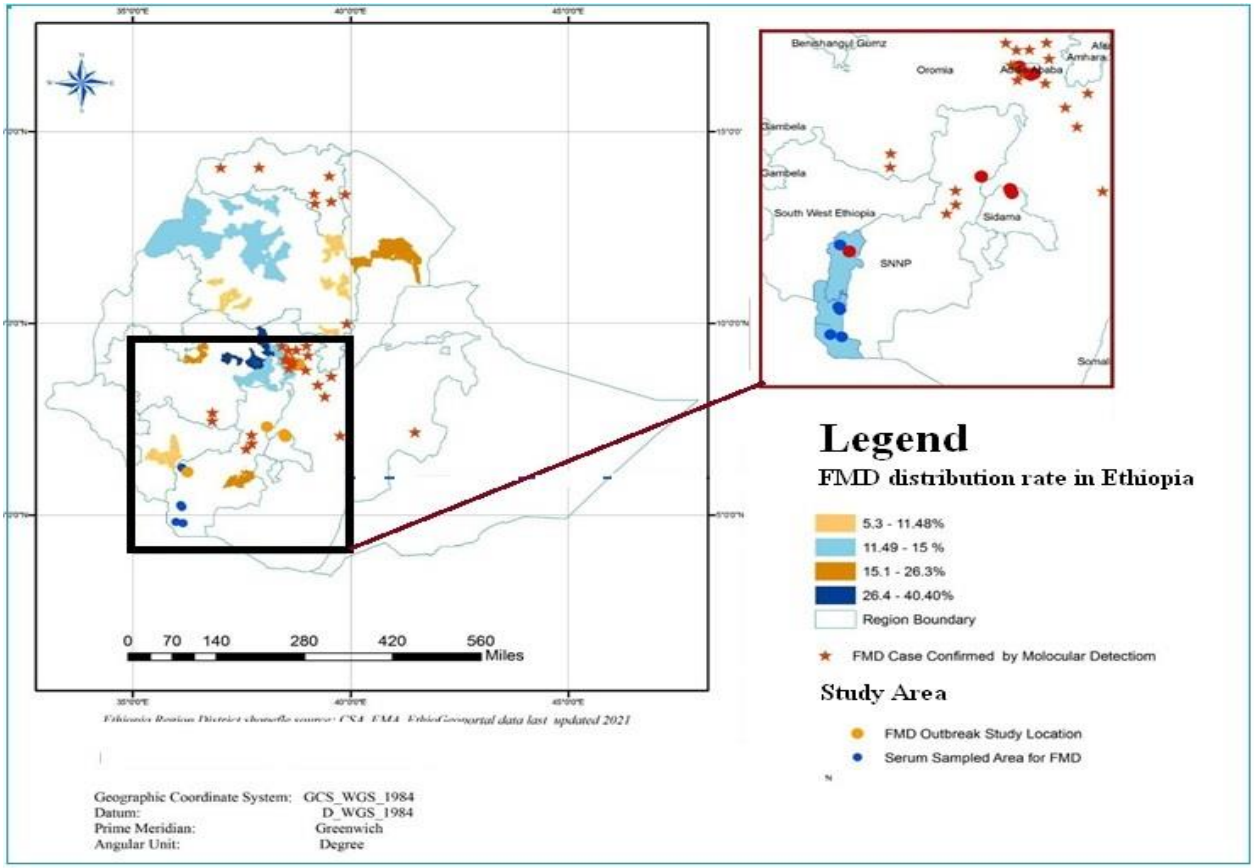


Figure 2: Map of the study area

Central Ethiopia's Halaba Kulito (1554–2149m elevation) exhibits moderate temperatures (12.8–26.8°C), contrasting sharply with South Omo Zone's arid lowlands (769 m elevation), characterized by semi-arid conditions, sporadic rainfall (390 mm/year), and temperatures of 18–32°C. These regions collectively highlight Ethiopia's climatic and topographic diversity, spanning highland plateaus, temperate urban zones, and arid lowland ecosystems.

3.2 Study population

All cattle in the study area currently manifested the clinical signs of FMD lesions were included in the study subjects. The major important clinical signs observed during the outbreak investigation were excessive salivation, mouth lesions consisted of erosions and ulcers on tongue and dental pad, whereas foot lesions comprised erosions on the inter digital space and the coronary bands.

All breeds, Age, sex and management systems (the intensive, semi extensive and extensive management) were included in the study. All animal's ages were determined by dental eruption age determination method (Torell *et al.,* 2003) and therefore, animals were categorized as young (<2 years), adults (2–5 years), and old (> 5 years). For seroprevalence determination in the South Omo zone, only asymptomatic cattle and unvaccinated animals against FMD were selected while in active Outbreak investigation areas only cattle with active clinical sign were included.

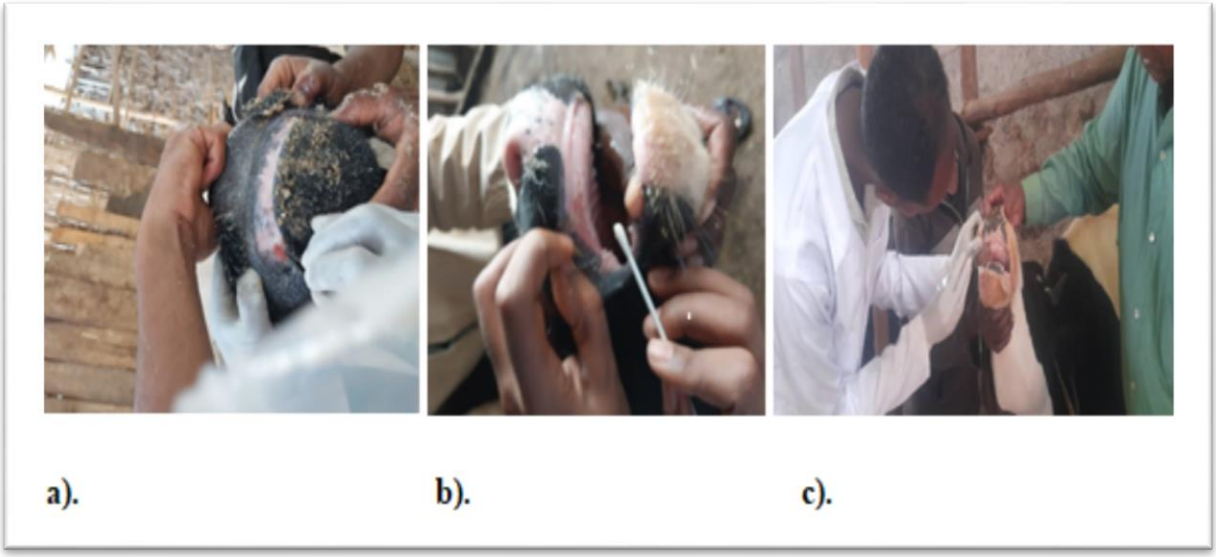


Figure 3: Sample collection from FMD infected and clinically active cattle

3.3. Study design

A cross sectional study and outbreak investigation was performed from October 2023 to May 2025.

3.4. Sample size determination

The sample size for the cross sectional study was determined using the Thrusfield formula (Thrusfield *et al., 2018*) based on Molla *et al.,* (2010) previous study of the disease's 8.18% seroprevalence in cattle in the South Omo zone with a 95% confidence level and 5% absolute precision. The sample size calculated, 115 for each depending on the seroprevalence reported previously as pointed above and sample number collected was increased to three fold of the calculated sample number in order to get accurate samples from the study area. The sample size required for the study was calculated based on the following formula:

$n = ([1.96]\ ^2 \times 0.08 \times (1 - 0.08))/〖0.05〗\ ^2 = 115 * 3 = 345$

Where n = sample size, Pexp = expected prevalence, and d = absolute precision.

Then, proportionate numbers of animals were sampled from the study area based on the cattle population size. But, during samples were collected depending on the calculated sample size from the concepts of previous study as stated above that only 314 animals' blood samples were gathered and 31 samples were missed due to not covering all this samples because of resources shortages like vacuatainer plain tubes at a time.

3.5 Sampling technique

The sample was collected purposively from selected areas by district, town, or locality based on the active disease outbreak, and serum samples were collected by using multistage random sampling method from South Omo Zone, in three districts from cattle that didn't manifest FMD clinical signs. Accordingly, in FMD active outbreak areas the tissue and swab samples were collected from towns or localities based on the active disease outbreak report. From each animal's manifesting the FMD clinical signs the epithelial tissue and swab samples was collected by using forceps and swab sticks, and kept in 2ml of viral transported media having the Phosphate buffer with antibiotics.

However, during the serum samples collection the proportional numbers of samples were collected depending on the numbers of cattle herd size categorized as small, medium and large herd sizes. The epithelial tissue samples and swab samples collected from the same locality or peasant association were collected as individual animal or pooled together in the same locality, and transported to virology laboratory of AHI, Sabeta.

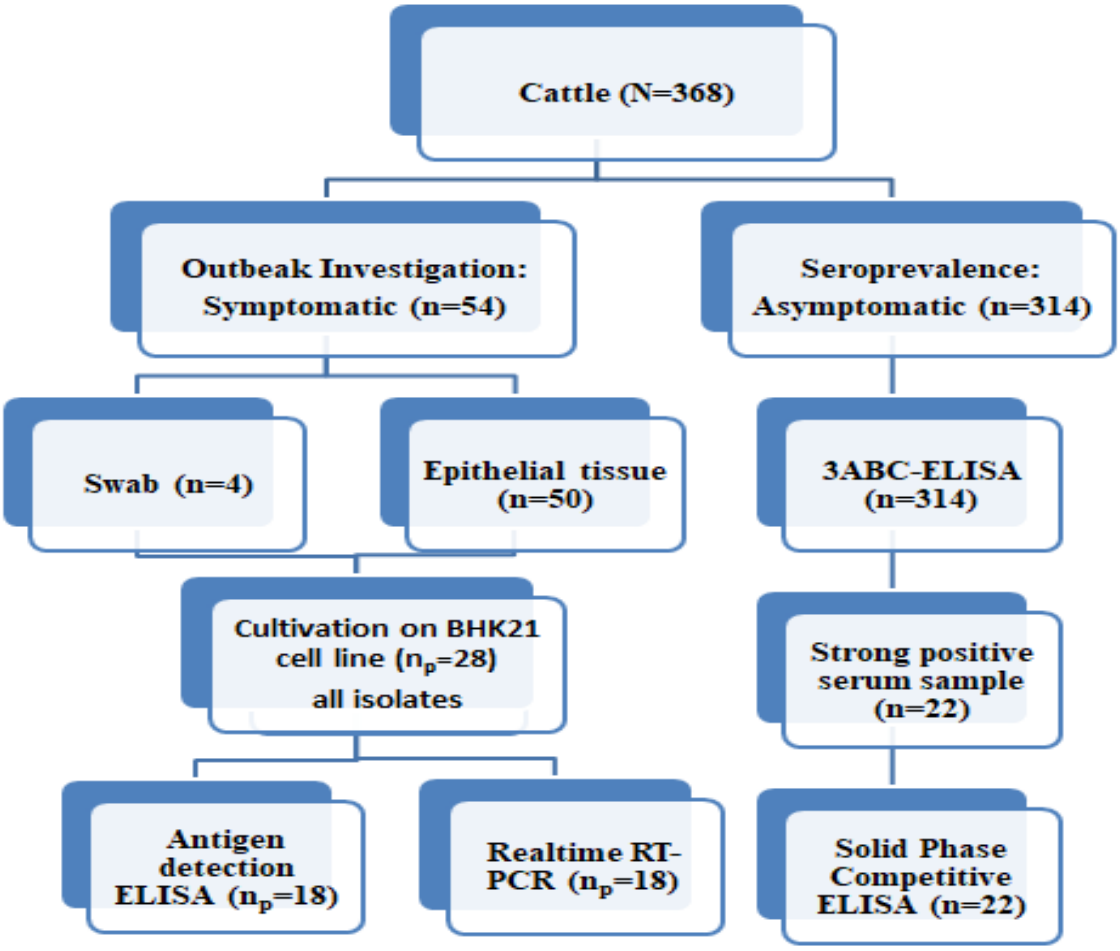
3.6 Sample collection and Transportation

Blood Sample Collection: From each sampled animal, 10 milliliters of blood were collected from the jugular vein using a needle and plain Vacutainer tubes under aseptic environment. The type of farm, the sampling location, the date, and code of identification were all listed on the label of each vacutainer tube. Following that, the serum samples were decanted into 2 milliliter sterile cryovials after being kept upright at room temperature over a night. Following that, the serum was transported to the Animal Health Institute (AHI), Sebeta, Ethiopia, using an icebox containing icepacks. Until being processed, the samples were kept at temperature of -20 °C.

Tissue Sample collection: Epithelial tissue samples were obtained from intact and freshly burst vesicles and placed in 0.04 M phosphate buffer with 50% glycerol. Therefore, based on the availability of active cases of FMD, tissue samples were gathered and preserved in virus-transported media (VTM) by placing the sample in the cooling icebox. Following labeling and preserved in icebox, the samples were transported to Animal Health Institute, Sebeta, where the samples were stored in a cold chain and held at -80 °C until processing.

3.7 Laboratory Technique

Multiple stage laboratory tests were applied to assess seroprevalence and antibody based serotyping and moreover, serotyping by using the *FMDV* antigen in outbreak investigation by using various samples such as serum, tissue and Swab samples according to the following study flow chart below.



NB: Where n_p = numbers of pooled sample purposively used during laboratory test, N=total numbers of animals sampled.

Figure 4: Laboratory techniques Flow chart used for *FMDV* isolation, detection and identification.

3.7.1. FMD Seroprevalence Study

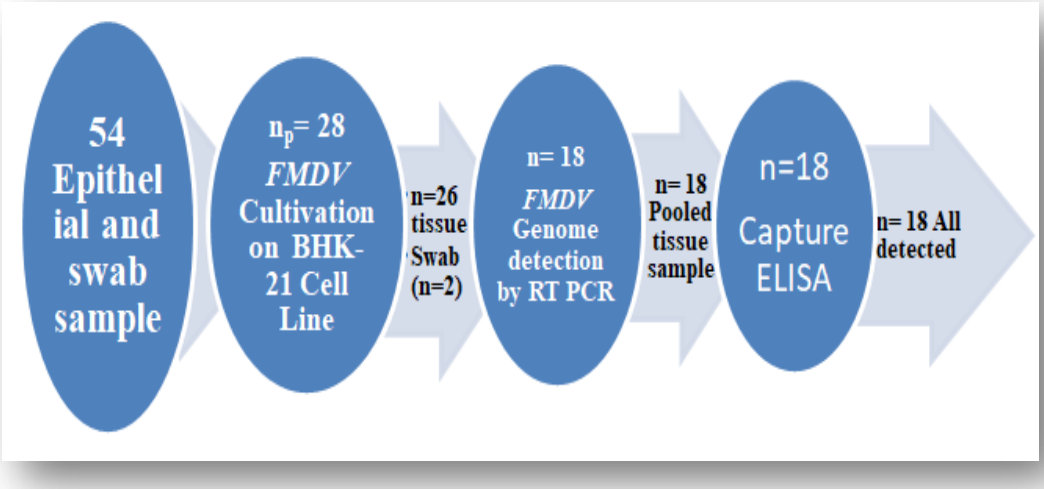
Competitive 3ABC-ELISA: The serum sample tested using competitive 3ABC ELISA (ID Screen® FMD NSP Competition, ID Vet, Grabels, France) in the Animal Health Institute in Sebeta, Ethiopia. The tests were carried out following the manufacturer's instructions, which were designated for detection of antibodies against FMDV nonstructural Protein (NSP) in Serum. Laboratory protocol procedure is indicated in Appendix 2. A spectrophotometer was used to measure the optical density (OD) at 450 nm. The test was considered validated if the mean value of the negative control OD was greater than 0.7 and the mean value of the positive control OD was less than 30% of the ODNC. Based on the competition percentage (S/N %)

for each sample, the interpretation was determined as follows: S/N% = OD sample/NC x 100. If S/N% value was less than or equal to 50% samples were defined as Positive, while if the S/N% value greater than 50% samples was negative to the virus.

Solid Phase Competitive-ELISA: Strong positive serum resulted against *FMDV* by Using 3ABC ELISA were serotyped by using Solid Phase Competitive-ELISA (ISZLER, Brescia, Italy) to identify specific Serotype found in each sample. The tests were carried out following the manufacturer's instructions as described Laboratory protocol procedure is indicated under Appendix 5. A spectrophotometer was used to measure the optical density (OD) at 450 nm. The test was considered validated if the mean value of the negative control OD was greater than 0.8 and the positive control serum is expected to give $\geq$90% inhibition at 1/10 dilution and >50% inhibition at the second dilution (1/30). This assay uses selected neutralizing anti-*FMDV* monoclonal antibodies (Mab) specific for *FMDV* serotypes to measure specific antibodies against the particular serotypes.

3.7.2 FMD Outbreak investigation

The tissue and swab samples were collected from animals exhibiting clinical signs of Foot and Mouth Disease (FMD) in five selected regions of Ethiopia in Addis Ababa (Akaki kality woreda 07 and 10) and Sheger city (Burayu), and the pooled tissue samples obtained from Habas, Hawassa city, and Halaba kulito town, and the pooled swab samples were taken from South Omo zone (Salamago district. These samples were subsequently processed using cell culture techniques with BHK21 cell lines to isolate the virus and identify the circulating serotypes of *FMDV* through antigen-captured ELISA. The *FMDV* genome was detected by using RT PCR after pooled samples based on their locality.



Where n= Sample, n_p =numbers of pooled Sample

Figure 5: Flow chart of FMD outbreak samples testing techniques

RNA Extraction and Detection of *FMDV* by real time RT-PCR: Following the manufacturer's instructions as stated by Abdelfattah *et al.*, (2022), the total RNA was extracted from supernatants of the homogenized epithelial tissue sample using the QIAamp RNA Mini kit (Qiagen, Hilden, Germany) and the laboratory procedure protocol for RNA extraction and *FMDV* genome detection by RTPCR was described in detail under Appendix 4. The extracted RNA samples (OIE, 2017) were tested by real time RTPCR technique, which targeted the 3D regions of the FMD virus genome to determine the presence of viral RNA.

For this RNA amplification purpose, the forward primer (5'-ACT GGG TTT TAC AAA CCT GTGA-3'), reverse primer (5'-GCG AGT CCT GCC ACG GA-3'), and the 3D probe (5'-6-FAM-TCC TTT GCA CGC CGT GGG AC TAMRA-3') were used (Cahallan *et al.*, 2022). After the PCR amplification was performed based on the instrument software (Applied Biosystems 7500 Real-Time PCR System, Software), the successfully amplified target presented an amplification curve and the cycle threshold (Ct) at which the target amplicon first appeared above the background fluorescence levels. The mean Ct value and standard deviation were given for each RTPCR, which was carried out minimally in duplicate (Mukasa *et al.*, 2016).

FMD Virus Isolation: Totally 28 epithelial tissue and swab samples collected from FMD active outbreak areas were cultivated on baby Hamster Kidney21 cell lines following the manufacturer's instructions described under Appendix 1, that 1 ml of filtered tissue suspensions were inoculated on confluent baby hamster kidney (BHK-21) monolayer cells. The cells were then incubated at 37 °C for 1 hour to allow the virus to absorb into the cells (OIE, 2009). Then, for three consecutive days, the CPE was regularly evaluated three times a day at intervals of twenty to 24 hours.

Antigen Detection Captured Sandwich ELISA: The tissue and swab samples resulted CPE positive after inoculated to confluent monolayer BHK21 cell lines were further serotyped to identify *FMDV* serotypes by using *FMDV* Antigen Detection and Serotyping ELISA (ISZLER, Brescia, Italy). The tests were carried out following the manufacturer's instructions as described Laboratory protocol procedure is indicated under Appendix 3 and the validation Criteria for antigen detection by sandwich ELISA result was interpreted based on manufacture manual optical density (OD) value.

3.8 Questionnaire survey

The sample size for Questionnaire survey was determined using the formula (n = 0.25/SE2) as per Arsham at the standard error (SE) of 0.05 with 95% confidence interval (Arsham, 2017). Accordingly, 100 interviews were conducted with respondents with South Omo, and 45 respondents were from the outbreak reported area. The semi- structure questionnaire developed originally in English then translated to seven local languages Me'en language, Daasanach language, and Nyangatom language, Sidama language, Halaba language, Afaan Oromo and Amharic language were used to collect community knowledge, Attitude and Practice toward to FMD assisted with KoboCollect tool.

3.9 Data management and Analysis

Data describing FMD information gathered both from field sample and laboratory studies were appropriately coded, cleaned and entered in to Microsoft Excel® 2013. The descriptive statistical method was used to compile the data. The data was then loaded into STATA statistical software version 14.0. The potential risk factors and

FMD seropositivity was analyzed by p-value that when the p-value was less than 0.05, the results were likely to be statistically significant after conducting binary logistic regression for risk factors and FMD seropositivity was analyzed by multi-variable binary logistic regression analysis. The questionnaire survey data collected from respondents' to assess the knowledge, attitude and practices was determined by descriptive statistics.

3.10. Ethical Approval

This ethical permission for this project was granted by Addis Ababa University College of Veterinary Medicine Animal Research Ethics Committee by specifying reference number VM/ERC/02/61/16/2025. Thus, all processes were conducted by qualified experts in accordance with the guidelines and standards established by the university's ethical committee. The well-being and proper care of the animals throughout the entire investigation were ensured during the research was conducted. All owners of dairy farm and pastoral animals' owners provided their verbal consent prior to the start of the study for the questionnaire interview as described under Appendix6, as well as for the collection of their animals' serum or blood, epithelial tissues, and swab samples. All the target owners of the animals were informed that they had the right to know their animals' laboratory tests outcomes.

4. RESULTS

4.1 Seroprevalence Result

FMDV antibodies were detected in 80.89% (254/314) of cattle tested. Seroprevalence varied significantly by district ($\chi^2 = 25.5$, $p < 0.001$) and herd size ($\chi^2 = 67.1$, $p < 0.001$), but not age ($p = 0.769$), sex ($p = 0.603$), or body condition ($p = 0.188$). Daasanech district had the highest seroprevalence (95.0%; 95% CI: 92.8–97.2%), exceeding Nyangatom (70.5%; 95% CI: 62.0–79.1%) and Salamago (74.4%; 95% CI: 65.0–83.8%). Prevalence increased with herd size: small herds 51.7% (41.2–62.2%), medium 89.1% (82.7–95.6%), and large herds 94.1% (90.1–98.0%) (Table 2).

However, no significant differences occurred among age groups: young (<2 years) 80.0% (72.3–87.7%), adults (2-5 years) 77.9% (70.5–85.2%), and older cattle (>5 years) 83.5% (75.5–91.6%). Seroprevalence was comparable between males (82.2%; 75.7–88.7%) and females (79.9%; 74.0–85.8%). Body condition showed a non-significant trend toward higher prevalence in poor-condition animals (85.7%; 77.0–94.4%) versus medium (80.6%; 75.0–86.3%) or good condition (76.7%; 65.7–87.6%) (Table 2).

However, no significant association was observed between body condition score and FMDV seroprevalence ($\chi^2 = 3.35$, $p = 0.188$). Nevertheless, seropositivity was numerically higher in cattle with poor body condition (85.7%; 54/63) compared to medium (81.0%; 154/191) and good condition (76.7%; 46/60). Similarly, age groups showed comparable seroprevalence patterns: young (<2 years) 80.0% (88/110), adult (2-5 years) 77.9% (95/122) and old (>5 years) 83.5% (71/85), with no statistical significance ($p = 0.769$).

Out of 254 *FMDV*-3ABC-antibody positive samples, 22 high-titer specimens were selected for serotyping via Solid-Phase Competitive ELISA (SPCE ELISA), which identified four serotypes: SAT2 (81.8%, 18/22), O (72.7%, 16/22), SAT1 (59.1%, 13/22), and A (45.5%, 10/22). Serotype distribution varied geographically, with all four serotypes detected in Daasanech (SAT2: 45.5% [10/22], O: 40.9% [9/22], SAT1: 27.3% [6/22], A: 22.7% [5/22]) and Nyangatom (SAT2: 31.8% [7/22], O: 22.7%

[5/22], A: 22.7% [5/22], SAT1: 18.2% [4/22]), while Salamago exhibited only three serotypes (O: 9.1% [2/22], SAT1: 13.6% [3/22], SAT2: 4.5% [1/22]). SAT2 demonstrated the highest overall prevalence and was the predominant serotype in both Daasanech and Nyangatom districts (Table 2).

Table 2: Overall seroprevalence-antibodies against *FMDV* and risk factors analysis.

Risk factors	**Categories**	**Number animals tested**	**Number positive**	**%**	**OR**	**95% CI OR**	**P-value**
Study area	Daasanech	120	114	95%	0.12	0.05-0.1	
	Nyangatom	112	79	70.5%	Ref.		0.000
	Salamago	82	61	74.4%	0.15	0.06-0.4	
Age	Young (≤2)	110	88	80%	1	0.53-1.9	
	Adult (2-5)	122	95	77.8%	Ref.		0.769
	Old (≥5)	85	71	83.5%	1.2	0.6-2.6	
Sex	Male	135	111	82.2%	1.1	0.65 – 2	
	Female	179	143	79.9%	Ref.		0.603
Body	Poor	63	54	85.7%	2	0.9-5.98	
Condition	Medium	191	154	81%	1.48	0.7-2.9	0.188
Score	Good	60	46	76.6%	Ref.		
Heard Size	Small	87	45	51.7%	Ref.		
	Medium	92	82	89%	4	2.1-7.7	0.000
	Large	135	127	94%	9	2.68-6.79	
Total		**314**	**254**	**80.9%**			

NB: 95% CI = ninety five percent of confidence interval; p- value = probability value; **$\chi 2$ = chi – squared value**.

Table 3: Serotyping of FMD virus from south Omo zone district

No.	District	Positive Sample (n)	Serotype O	Serotype A	Serotype SAT1	Serotype SAT2
1	Salamago	6	(2/22)	0	3	1
2	Daasanech	10	(9/22)	5	6	10
3	Nyangatom	6	(5/22)	5	4	7
	Total	**22**	**72.72% (16/22)**	**45.45% (10/22)**	**59% (13/22)**	**81.81% (18/22)**

4.2 Logistic regression analysis

4.2.1 Univariable logistic regression analysis of the putative risk factors

Table 4 and 5 below shows that univariable logistic regression revealed the existence of risk factors that influence the seroprevalence of antibodies against FMDV. As a result, the recent study revealed the risk factors that most likely did not affect the cattle seropositivity of antibodies against FMD in possible risk factors like age (p = 0.769), body conditions (p = 0.188) and sex (p = 0.603), and were among the variables removed from the model using univariable logistic regression analysis, with a p-value of 0.25. Univariable logistic regression analysis by this study ensured that the risk factors like study area (OR = 0.5526804; 95% CI = 0.3615221- 0.844915, p = 0.000) and herd sizes (OR = 5.185512; 95% CI =3.21691 - 8.358807, p = 0.000) were significantly associated ($p < 0.05$) with seropositivity of antibody against FMDV as shown in Table 4 below.

Table 4: Univariable logistic regression analysis of risk factors.

Explanatory variable	OR	95% CI	P-value
Study area	0.5526804	0.3615221- 0.8449155	0.006
Age	1.05992	0.7006367 - 1.603442	0.783
Sex	0.7636238	0.3940363 - 1.479867	0.424
Body condition	0.6841801	0.4158266 - 1.125716	0.135
Herd size	5.185512	3.21691 - 8.358807	0.000

OR = Odd ratio, Ref. = References CI = Confidence interval, Young = age less than 2 years, Adult = age between 2 to 5 years and Old = age ≥ 5.

4.2.2 Multivariable logistic regression analysis of the putative risk factors

The disease incidence in Dasenech district was almost 0.125 times more likely higher (OR = 0.125; 95%CI= 0.05-0.31) than the disease incidence in Nyangatom. Hence, statistically the seropositivity of antibodies against FMDV was significantly higher (p = 0.000) in large herd size than the small herd size. The disease incidence in Salamago district was 0.15 (95% CI = 0.06-0.4) times higher than the disease incidence in Nyangatom district. There was highly significant difference (p = 0.000) between the large, medium and small herd sizes with higher disease incidence in large and medium herd size than the small herd size. However, the disease incidence in the large herd was the highest prevalence that 9 times (95% CI =2.68-6.79) higher than the disease incidence in small herds. The disease prevalence in medium size herd was 4 times (95% CI = 2.13-7.7) higher than the disease incidence in the small herd size.

Table 5: Multivariable logistic regression analysis of potential risk factors.

Risk factors	Categories	OR	(95% CI of OR)	p - value
Study area	Salamago	0.12	0.05-0.1	0.692
	Dasenech	0.15	0.06-0.4	0.000
	Nyangatom	Ref.		
Herd size	Small	Ref.		
	Medium	4	2.13-7.7	0.000
	Large	9	2.68-6.79	0.000

OR = Odd ratio, Ref. = References CI = Confidence interval, Chi 2 = chi-squared.

4.3 Outbreak Investigation Result

Virus Isolation: Cytopathic effect (CPE), indicating successful isolation of live *Foot and Mouth Disease Virus (FMDV),* was observed in 18 of 28 clinical samples (64.3%) inoculated onto BHK21 cells. Geographical analysis revealed complete positivity in samples from Akaki Kality-7 (8/8) and Burayu (3/3), whereas samples from Hawassa City (0/1) and Salamago (0/2) yielded no isolates.

Real-Time RT-PCR: The 18 CPE-positive virus isolates were subsequently tested using universal *FMDV* primers and probes. Viral RNA was detected in 10 isolates (55.6%), exhibiting Ct (Cycle threshold) values ranging from 19.8 to 32 (Fig. 5a-c). Positives originated from: Akaki Kality (Districts 07 & 10; n=4), Habas District (n=2), Halaba Kulito Town (n=2), and Burayu Town (n=2).

Serotyping analysis of virus isolates (n=18) from active FMD outbreaks confirmed the co-circulation of serotypes O and A. Serotype O was dominant, identified in **66.7% (12/18)** of isolates, followed by serotype A in **16.7% (3/18)**. Mixed O/A infections were detected in **16.7% (3/18)** of isolates. Geographically, serotype O outbreaks occurred in Akaki Kality (predominantly District 07; 44.4%, 8/18 isolates), Burayu (16.7%, 3/18), and Halaba Kulito (5.6%, 1/18). Serotype A outbreaks were localized to Habas District (11.1%, 2/18) and Halaba Kulito (5.6%, 1/18). Mixed O/A infections were exclusively associated with Akaki Kality District 07 (16.7%, 3/18). No *FMDV* was isolated from samples originating in Hawassa City or Salamago District (Table 7).

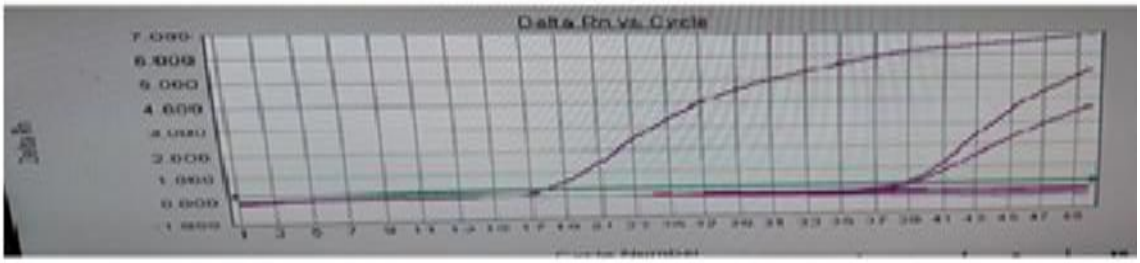


Figure 5A: Swab sample for FMD virus detection from Salamago district of South Omo zone Result

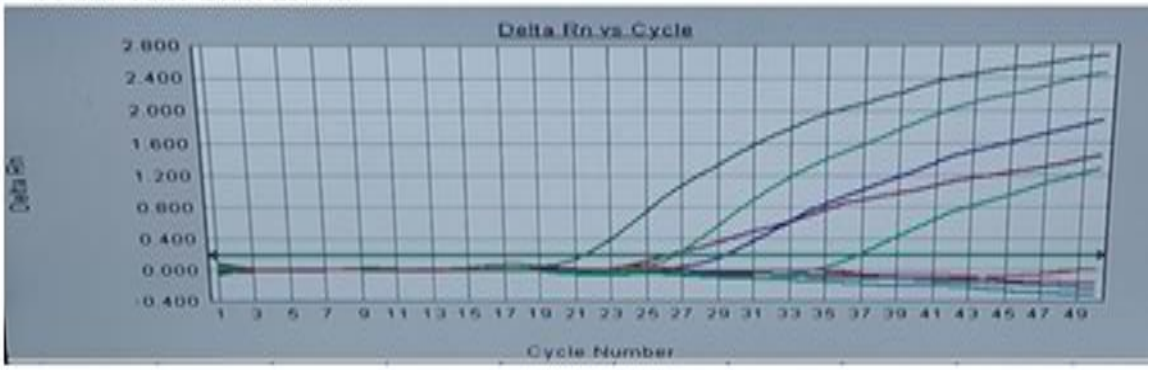
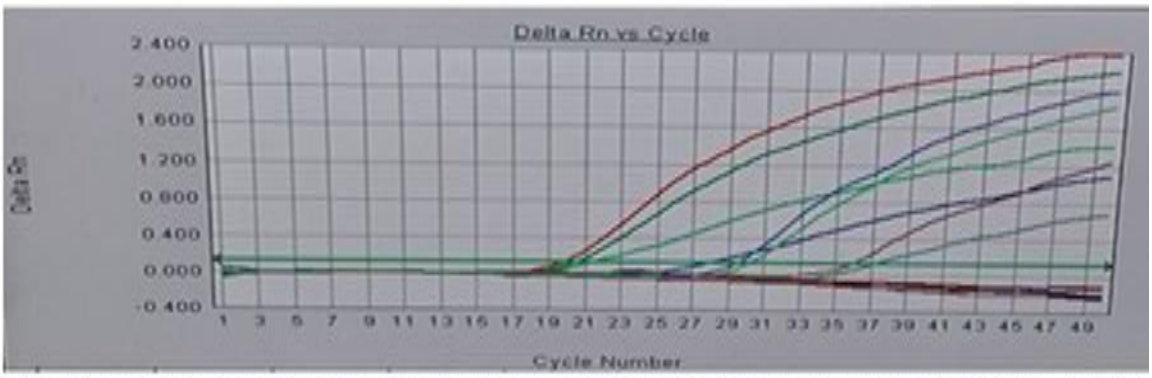


Figure 5B:Tissue sample for FMD virus detection from Hawassa city, Habas district and Halaba Kulito town Result



The Figure 5C: Tissue Sample for FMD virus detection from Burayu district, Addis Ababa Akaki Kality woreda 07 and woreda 10 result

Figure 6: Real-time RT-PCR Positive results indicating amplification curve (above threshold line).

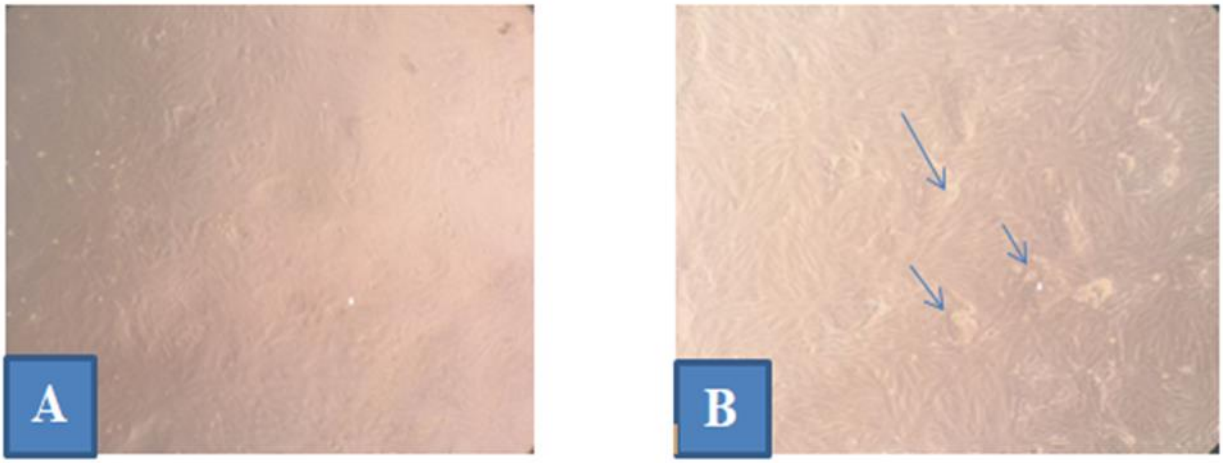


Figure 7: *FMDV* CPE result on BHK21cell line.

Figure 7A indicates the BHK21 Normal confluent cell layer before the virus and Figure 7B describes the *FMDV* CPE positive on BHK21 cell lines with syncytium formation.

Table 6: *FMDV* virus isolation, serotyping & Molecular detection result.

No.	Region	District	Sample Number	BHK21 CPE	Serotypes			RT-PCR (n=18) Pooled Sample
					O	A	Mixed O&A	
1	**Addis Ababa**	Akaki kality-7	8	8	5	0	3	3
		A/kality-10	5	3	3	0	0	1
2	**Oromia**	Habas	4	2	0	2	0	2
		Burayu	3	3	3	0	0	2
3	**Central Ethiopia**	Halaba kulito	5	2	1	1	0	2
4	**Sidama**	Hawassa city	1	0	0	0	0	0
5	**South Ethiopia**	Salamago	2	0	0	0	0	0
	Total		**28**	**18**	**12**	**3**	**3**	**10**

4.4. Questionnaire survey result

Respondent Demographics

A semi-structured questionnaire assessing knowledge, attitudes, and practices (KAP) related to Foot and Mouth Disease (FMD) was administered to 145 respondents across eight districts in South Ethiopia's South Omo Zone and reference sites. Geographic representation included Salamago (21.4%, 31/145), Daasanech (23.4%, 34/145), and Nyangatom (24.1%, 35/145), with smaller proportions from Addis Ababa Akaki Kality (6.9%, 10/145), Burayu (4.1%, 6/145), Habas (6.9%, 10/145), Halaba Kulito (9.0%, 13/145), and Hawassa City (4.1%, 6/145). Respondents were predominantly male (96.6%, 140/145) and married (97.2%, 141/145). Age distribution followed standardized categorizations (Toossi, 2011; Achew *et al.*, 2018), with 81.4% (118/145) aged 30–60 years, 11.7% (17/145) under 30 years, and 6.9% (10/145) over 60 years. Occupations were dominated by pastoralists (43.4%, 63/145), followed by smallholder farmers (25.5%, 37/145), dairy farmers (23.4%, 34/145), and veterinary professionals (7.6%, 11/145). Education levels revealed significant illiteracy (60.7%, 88/145), with 24.1% (35/145) attaining elementary education, 6.9% (10/145) completing high school, and 8.3% (12/145) holding degrees.

Farmer Knowledge of Foot and Mouth Disease

Survey results revealed widespread awareness of FMD among respondents, with 81.4% (117/145) reporting familiarity with the disease, while 19.3% (28/145) indicated no prior knowledge. However, critical gaps existed in biosecurity understanding: only 4.8% (7/145) recognized the need for vaccination or quarantine of infected animals, contrasting sharply with 95.2% (138/145) who acknowledged neither practice. Symptom recognition was more prevalent, with respondents identifying FMD primarily through excessive salivation (26.2%, 38/145*), oral/udder lesions (27.6%, 40/145*), and lameness (32.4%, 47/145*). Notably, 14.5% (21/145) could not describe any clinical signs. Regarding outbreak timing, 17.2% (25/145) reported occurrences within the preceding 6 months, while 67.6% (98/145) indicated outbreaks beyond this period. Perceived transmission routes included animal markets (38.6%, 56/145), wildlife contact (18.6%, 27/145), and neighboring herd exposure

(23.4%, 34/145), with minor contributions (1.4%, 2/145) attributed to other sources (Table 8).

Table 7: The overall Respondent demographic information on questionnaire survey.

Variables	Category	Response/Total	Percent (%)
Study area	Salamago	31	21.5%
	Daasanech	34	23.5%
	Nyangatom	35	24%
	Addis Ababa Akaki kality	10	7%
	Burayu	6	4%
	Habas	10	7%
	Halaba kulito	13	9%
	Hawassa city	6	4%
Gender	Male	140	96.5%
	Female	5	3.5%
Age	Age less than 30 years	17	12%
	Age between 30 – 60 years	118	81%
	Age above 60 years	10	7%
Marital status:	Married	141	97%
	Single	4	3%
Occupation	Small holder farmers	37	25.5%
	Employee	11	7.6%
	Pastorals	63/145	43.4%
	Dairy farmers	34/145	23.4%
Level of education:	Illiterate	88/145	60.%
	Elementary	35/145	24%
	High School	10/145	7%
	Degree	12/145	8.3%

Table 8: The Knowledge of community to ward FMD.

Categories	Response	Frequency	Percent (%)
Knowledge FMD	Yes	117/145	81%
	No	28/145	19%
Vaccinating the animals	Yes	(7/145)	5%
	No	(138/145)	95%
The know-how animals quarantining	Yes	(7/145)	5%
	No	(128/145)	95%
Symptoms of FMD	Excessive salivation	38/145	26%
	Lesions and Ulcers on tongue, gums and udder	40/145	27.5%
	Lameness	47/145	32.5%
	No	20/145	14%
Time of FMD occurrence.	Before six months	25/145	17%
	Less than six months	100/145	67%
	No	20/145	14%
The means of FMD transmission	From market animals entered to herd	55/145	38%
	From neighboring animals	34/145	23%
	From wild life contact	26/145	18%
	No	20/145	14%
	Others suspicion (contact of animals with virus contaminated feed and workers materials)	10/145	1%

Community Practices and Attitudes toward Foot and Mouth Disease

Survey responses revealed heterogeneous approaches to FMD management during outbreaks. While 34.5% (50/145) of respondents reported cases to veterinary professionals, most employed alternative strategies: 22.8% (33/145) treated animals directly, 13.8% (20/145) utilized traditional remedies, and 10.3% (15/145) undertook no intervention. Critical knowledge gaps persisted, with 13.8% (20/145) unable to recognize clinical signs. Notably, only 4.8% (7/145) implemented quarantine measures to separate infected animals. No respondents reported selling affected cattle to village butchers as a control strategy (Table 7).

Table 9: Community attitudes and practices toward FMD Control and Prevention

Practice	**Categories**	**Response/ Total**	**Percent (%)**
What would you do if you suspected that your herd had Foot and Mouth Disease?	Quarantining the diseased animals	7/145	5%
	Report to veterinary Professionals	50/145	34%
	Treating the animals	33/145	23%
	Do nothing	15/145	10%
	Sell cattle to village butcher	0	0%
	No	20/145	14%
	Others/traditional treatment	20/145	14%

5. DISCUSSION

This study investigated Foot and Mouth Disease (FMD) outbreaks and assessed FMD seroprevalence in cattle across selected districts of South Omo Zone, South Ethiopia. Using the 3ABC-NSP ELISA, we detected anti-*FMDV* antibodies in 80.89% (254/314) of purposively collected serum samples from Salamago, Daasanech, and Nyangatom districts. This finding represents a notably high level of infection, exceeding most previous seroprevalence reports from Ethiopia.

Specifically, the observed seroprevalence (80.89%) is higher than the 72.1% reported by Awel *et al.* (2021) in central Ethiopia and significantly surpasses several other regional studies: Shurbe *et al.* (2022) reported 26.82% in Gamo Zone; Ahmed *et al.* (2020) found 40.4% in West Shewa; Bandaw *et al.* (2024) reported 46.88% in Wolaita Zone; Seifu *et al.* (2023) found 56% around Addis Ababa; and Megersa *et al.* (2009) reported only 9.5% in Southern Ethiopia. However, our result aligns closely with the 83.8% seroprevalence reported by Nthiwa *et al.* (2020) in the Maasai Mara, Kenya. This similarity may reflect shared ecological and management factors, notably extensive pastoral systems and significant wildlife-livestock interactions in both regions.

The co-circulation of multiple serotypes (O, A, SAT1, SAT2) resembles patterns observed in Chad by Ouagal *et al.* (2018) and Kenya by Nthiwa *et al.* (2020), where similar environmental conditions (high rainfall/humidity) and transboundary animal movements in search of grazing were implicated. In contrast, Seifu *et al.* (2023) identified only serotypes A and O in dairy cattle around Addis Ababa and Sabeta. The high overall seroprevalence observed was statistically significant across the districts ($p = 0.000$), consistent with earlier findings by Molla *et al.* (2010) in the South Omo Zone. Key risk factors likely contributing to this elevated prevalence include dynamic wildlife movements and frequent contact between wildlife and cattle, particularly during shared grazing and watering, cross movements to National parks and sanctuaries, and dynamic animals (domestic ruminants) movements throughout the districts because of the search for fodder (Gebeyehu *et al.,* 2021).

District-level analysis revealed significant variation in seroprevalence: Daasanech had the highest rate (95%), followed by Salamago (74.39%) and Nyangatom (70.53%). The prevalence in Salamago and Nyangatom aligns with the 71.4% reported by Awel *et al.* (2021) for Holeta, while the exceptionally high prevalence in Daasanech mirrors the 97% observed in Ada'a Barga by the same study.

Herd size emerged as a significant risk factor ($p = 0.001$). Prevalence increased markedly with herd size, reaching 94% in large herds. This strong association is likely driven by increased animal density and contact frequency, facilitating *FMDV* transmission. This finding is consistent with studies by Tegegne *et al.* (2024) and Batu *et al.* (2025) in Jimma Zone, Dubei and Negash (2021) in the Afar region, and Woldesenbet *et al.* (2023) in Boset and Adama districts.

In contrast, intrinsic factors such as sex and age were not significantly associated with seroprevalence ($P > 0.05$). Seroprevalence was similar between male (82.11%) and female (79.88%) cattle. This lack of association concurs with previous findings by Woldesenbet *et al.* (2023), Yahya *et al.* (2013), and Mohamoud *et al.* (2011).

The odds of being seropositive of the cattle to FMDV is 0.125 times higher in Dasenech district than the Nyangatom district indicates the higher disease incidence was occurred in the Dasenech was due to the presence of uncontrollable cattle movement around the Omo delta for the search of seasonal fodder. These risk factors was actually strongly agreed with study conducted by Molla *et, al,* (2010), that the high seroprevalence of the animals with in Dasenech was due to the uncontrollable animals movements crossing the boundary of the national Parks and Sanctuaries which might made great intermingling of wild animals and herds from different areas because for the search of fodder. Likewise, the odd of being seropositive of the cattle to FMDV is 0.15 times in Salamago district when compared with the Nyangatom district which indicates that the disease incidence Salamago was higher than the disease incidence in Nyangatom. The odds of being seropositive of the cattle to FMDV are 9 times higher in large herd size than cattle with small herd size, and 4 times in medium herd size than the small herd size.

Solid Phase ELISA identified four concurrent *FMDV* serotypes circulating in the study area: SAT2 (81.81%), O (72.72%), SAT1 (59%), and A (45.45%). This co-circulation of multiple serotypes (O, A, SAT1, SAT2) resembles patterns observed in Chad by Ouagal *et al.* (2018) and Kenya by Nthiwa *et al.* (2020), where similar environmental conditions (high rainfall/humidity) and transboundary animal movements in search of grazing were implicated. In contrast, Seifu *et al.* (2023) identified only serotypes A and O in dairy cattle around Addis Ababa and Sabeta.

Outbreak investigation using real-time RT-PCR and antigen capture sandwich ELISA on tissue and swab samples confirmed the presence of *FMDV* genome and antigen. Outbreak diagnostics specifically identified serotypes O and A, including mixed infections: serotype O was detected in 66.7% of positive samples, serotype A in 16.7%, and mixed O/A infections in the remaining 16.7%. This finding confirms previous reports of serotypes O and A circulating in Ethiopia. Serotype O was detected by Awel *et al.* (2021) in Addis Ababa and Ada'a Berga; Tesfaye *et al.* (2021) in Addis Ababa and Bishoftu; and Suleyaman *et al.* (2018) in central Ethiopia. Serotype A was reported by Ayelet *et al.* (2009) in Oromia, Tigray, Dire Dawa, and SNNPR, and by Negessu *et al.* (2023) in Dima Guranda and Sabeta. Mixed O/A infections were observed by Negessu *et al.* (2023) and Woldesenbet *et al.* (2023) in Boset and Adama.

Serotype O was the dominant strain in this outbreak (66.7%), aligning with several Ethiopian studies reporting high prevalence: Tesfaye *et al.* (2023): 60% in Tigray; Negessu *et al.* (2023): 87.5% in Central Ethiopia; Dubei and Amare (2020): 100% in Afar; Awel *et al.* (2009): 90%; Seifu *et al.* (2023): 75.5% in Central Ethiopia. Tesfaye *et al.* (2020) also reported serotype O dominance in Northern and Central Ethiopia. The prevalence of serotype A (16.7%) and mixed O/A infections (16.7%) in this study aligns partially with prior work: Negessu *et al.* (2023) reported 25% serotype A, while Tesfaye *et al.* (2020) reported 18% serotype O (lower than this study), 46% serotype A (higher), and 2.33% mixed O/A (lower).

The questionnaire survey revealed that 81% of respondents were familiar with Foot and Mouth Disease (FMD), primarily identifying it through clinical signs. This aligns with studies by Seifu *et al.* (2023; 90.2%), Tegegne *et al.* (2024; 86%), and Mesfine *et al.* (2019; 82.4%), confirming broad awareness among pastoral and dairy farmers.

Respondents accurately described clinical manifestations, including excessive salivation, oral/hoof lesions, and lameness consistent with observations by Barre *et al.* (2024) in Somalia. Notably, FMD is recognized by diverse local terminologies: "Maassaa" (Afaan Oromo), "Masa" (Sidama), "Maza" (Halaba), "Butlich" (Bodi/Me'en), "Ekire" (Nyangatom), and "Wuluf" (Daasanech), underscoring its endemicity across cultural contexts.

Fewer respondents (38%) identified market-sourced infected animals as the primary transmission route, a finding partially supported by Tegegne *et al.* (2024; 82%) but contrasting with Mesfine *et al.* (2019; 22%). Additionally, 23% attributed transmission to inter-herd contact during communal grazing, corroborating Mesfine *et al.* (2019; 78%) and Seifu *et al.* (2023; 57%), though diverging from Tolawak *et al.* (2023; 100%). Secondary routes included wildlife contact (18%, especially in South Omo) and contaminated feed/fomites (1%), the latter reinforcing Mesfine *et al.* (2019) on sporadic Amhara outbreaks linked to feed and contaminated objects.

Only 45% of respondents reported outbreaks to veterinary professionals. Disease occurrence peaked within six-month intervals (67%), consistent with Nyaguthii *et al.* (2019) in Kenya, where 5.9% of farmers reported recent FMD cases. Despite this, prevention measures were scarce: vaccination and quarantine coverage was minimal (5%), starkly contrasting with Athambawa *et al.* (2021) in Sri Lanka, where 49.2–54.5% of farmers practiced vaccination. Furthermore, 35% notified animal health professionals about infections, differing from Jemberu *et al.* (2015), where 78% of pastoralists reported annual outbreaks.

Furthermore, the limitations of the study was collecting samples only from Cattle species rather than including the small ruminants and Pigs because they are acted as the virus reservoir and others primarily susceptible to the disease to increase the disease transmission and incidence in the animals population. In addition, to increase the result of study more precise the collected sample size was not enough. For further characterizing the virus strain or topotypes further sequencing and constructing the phylogenetic tree was the main concept to be added and the main limitations of this study.

6. CONCLUSION AND RECOMMENDATIONS

This study confirms the hyperendemic status of Foot-and-Mouth Disease (FMD) within Ethiopia's South Omo Zone, evidenced by an alarming overall seroprevalence of 80.89%. Key drivers include large herd sizes and significant variability in infection rates across districts. The co-circulation of four serotypes (SAT2, O, SAT1, and A) significantly heightens transmission risks, further amplified by wildlife-livestock interactions and communal grazing practices. Active outbreaks were predominantly caused by serotype O (66.7%), with a notable proportion involving mixed O/A infections (16.7%). Despite high community awareness of FMD, critical gaps in control measures persist: only 5% of respondents implemented vaccination or quarantine, and fewer than 45% reported outbreaks to veterinary authorities. These findings collectively highlight an urgent need for serotype-specific interventions aligned with Ethiopia's National Livestock Master Plan (2021–2030) to mitigate both the disease burden and its significant impact on trade.

Based on these findings, the following targeted interventions are recommended:

- Serotype-Tailored Vaccination: Prioritize the deployment of trivalent (O, A, SAT2) vaccines rigorously matched to circulating local strains. This strategy is critical given the high SAT2 seroprevalence (81.8%) and the significant occurrence of mixed O/A infections (16.7%). Urgently improve vaccination coverage significantly beyond the current estimated 15%.

- Enhanced Surveillance and Diagnostics: Establish sentinel surveillance sites within key pastoral migration corridors. Implement real-time RT-PCR and virus isolation capabilities at these sites for rapid strain identification and outbreak response. Integrate systematic wildlife health monitoring to track potential spillover events and reservoir dynamics.

- Strengthened Community Engagement: Develop and implement culturally tailored community-based control programs. Train pastoralists in practical biosecurity measures (e.g., effective quarantine protocols, risks associated

with market-sourced animals) using local languages and culturally resonant terms (e.g., Maassaa, Masa).

- Targeted Research: Investigate the ecology and maintenance of SAT serotypes (particularly SAT2 and SAT1) within wildlife reservoirs. Conduct ongoing evaluation of field vaccine efficacy against the full spectrum of currently circulating strains to ensure intervention effectiveness.

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8. APPENDICES

Appendix 1: Virus Isolation.

The virus isolation steps conducted in the virus isolation room will be done accordingly:-

1) The epithelium tissue was taken from PBS/virus transport media and blotted dry on absorbent paper to reduce PBS solution and weighed.
2) The tissue sample will be grinded in small volume of tissue culture medium containing antibiotic (Penicillin (1000 IU [international unit])), Neomycin sulphate [100 IU], Polymycin B sulphate [50 IU], Mycostatin [100 IU], using sterile sand, Pestle and mortar and diluted in the same medium to a 10% suspension which centrifuged at 2000 rpm for 10 minutes and filtered through a membrane filter with 0.22µM pore size.
3) About 1ml of filtered tissue suspension was inoculated in confluent BHK-21 at media preparation, cell re-seeding and then thaw freezing.
4) The cell cultures will be added proportional amount of milliliters of the wanted maintenance medium (2% GMEM) and incubated at 37°C with 5% CO2 in humidified incubator.
5) The cell culture will be examined after 24 hours to a maximum of 72 hours, and the CPE positive cell cultures will be harvested at 85% CPE.
6) The cell cultures without CPE after 72 hours will be passages harvested and passages will be done to new monolayers as explained above.
7) Samples without or not developed CPE during the two passages will be considered virus negative sample.

Appendix 2: 3ABC-ELISA (DIVA Test)

The steps ought to be taken during ABC-ELISA technique of the result test for the presence of antibody against FMD will be listed below:-

1) The 96-well plate with the test and control specimens before transforming them to into an ELISA micro-plate y using the multichannel pipette.
2) The wash concentrate (20X) come to the room temperature in order to be solubilized.
3) The wash solution (1X) will be prepared by diluting the Wash concentrate (20X) to 1:20 in distilled or deionized water.
4) All reagents will be come to the room temperature and homogenized by using vortex.
5) For all protocols used steps:- 100µl of conjugate 1X to each well
6) The plate will be covered and incubated for 30 minutes ±3 min at 21°C (±5°C)
7) All well will be emptied and each well will be washed 5 times with at least 300 µl of the Wash Solution. And avoiding dryness of the wells will be done between each wash.
8) 100 µl of the substrate solution will be added to each well
9) The plate will be covered and incubated for 15 min ±2 min at 21°C (±5°C) in the dark
10) 100 µl of the Stop solution to each well will be added in the same order of the step 9 above.
11) The result outcome will be read and recorded under the O.D at 450 nm.

Appendix 3: Antigen Captured Sandwich ELISA

1) All solutions should be equilibrated in the laboratory before use as indicated on the manufacturers' guidelines paper and accordingly Washing solution (1X) will be prepared by diluting the PBS-Tween 10X concentrated 1/10 in distilled water.
2) Ten samples will be prepared and will be tested in one ELISA micro-plate
3) One positive inactivated control for each of FMDV types O,A,C,Asia1, SAT1, SAT2 and a negative control will be included in each plate that all these controls are incorporated into the ELISA micro plate trapped by respective catching Mab and by pan-FMDV Mab
4) Samples will be diluted into ½ ratio in diluent buffer
5) 50 µl for each well of each sample in 8 wells of column.
6) The 50µl diluent buffer will be distributed to all wells of 11 and 12 columns.
7) The plates will be incubated for 1 hour at room temperature (18 - 30°C)
8) The wells will be emptied and tap water will be hardly tapped to remove all remaining fluids
9) The wells will be filled with 200 µl/well of washing solution and incubated for 3mins at room temperature
10) The wells will be emptied and the steps will be repeated twice (three washing in total)
11) The residual fluids from plates will be tapping firmly onto a clean absorbent paper or towel
12) 50 µl of diluted conjugates/well will be added: conjugate A into rows from A to F and conjugate B into rows G and H
13) The plates will be covered and incubated for 1h at room temperature
14) The plates washed four cycles as above and leaving the last or 5^{th} wash for 5minutes
15) He chromogen substrates solution will be distributed 50 µl/well of the substrate/chromogen solution (equilibrated at room temperature) to all wells
16) Cover the plate and leave at room temperature for 20 minutes in the dark, starting timing when the first well is filled.
17) The reaction will be stopped by adding 50 µl/well of the stop solution following the same order used for the substrate solution. And mix the well content prior to reading.
18) Immediately after blocking each well will be read at 450nm wavelength using a micro plate reader. The validation Criteria for antigen detection by sandwich ELISA: The result will interpret based on manufacture manual optical density (OD).

Appendix 4: RNA extraction and real time RT-PCR technique FMDV genome detection

1. The work of RNA extraction was started by four Lysis steps that 560µl of mix solution (lysine solution buffer of = n sample *560µl and n* 5.6µl Carrier RNA) into sterilized centrifuge tube.
2. The swab specimen fluid will be vortex
3. 140µl of sample will be added to micro centrifuge tube containing Carrier RNA and lysine solution
4. Vortex for second to mix and incubate at room temperature
5. To makes binding 560µl absolute alcohol (Ethanol) to a centrifuge tube containing Lysine solution carrier of RNA and sample
6. Vortex for 1 second and bring Q Amp mini spin column with silica membrane
7. 630 µl from total solution to Q Amp mini spin column
8. Centrifuged at 8000rpm for 1 min
9. Discard the fluid and takeout the Q Amp mini spin column and keep it in a clean 2ml collection tube
10. The step 7 to 9 above will be done again with transferring the column filter to another or new collection tube
11. 500 µl wash buffers AW-1 in Q Amp mini spin column
12. Centrifuged at 8000rpm for 1 minute
13. Discard the fluid and transfer column filter to a new collection tube
14. 500 µl of wash buffers AW-2 in Q Amp mini spin column
15. Centrifuge at 1400rpm for 3min
16. Discard the flood and transfer column filter to a new collection tube
17. Centrifuge at 1400 for 1min to dry
18. 60 µl of elution buffer and incubate at room temperature for 1-3 min
19. Centrifuged at 8000ml for 1min to elute RNA from the Q Amp mini spin column
20. Remove Amp mini spin column and the elute RNA was stored at -80°C until additional utilize
21. The amplification cycles have three steps that Placing the plate in a real-time PCR machine for PCR amplification and run the following programme:- first cycle will be at 50°C for 2 minutes: 1 cycle; second at 95°C for 10 minutes: 1 cycle; and third cycle at 95°C for 15 seconds, 60°C for 1 minute: 50 cycles.

22. The CT values used to assign samples as either FMDV positive or negative test samples and negative controls will have a CT value at >50.0. Positive test samples and positive control samples should have a CT value <40 and the samples with CT values falling within the range 40–50 will be designated "borderline" and can be retested. Strong positive FMD samples have a CT value below 20 and the samples will be expected positive at CT value <36.

Appendix 5: SOLID PHASE COMPETITIVE-ELISA

The Test procedure for Solid phase ELISA will be:-

1) ELISA plates will be coated with 50µl/well rabbit antiserum homologous to the antigen being used, diluted in carbonate/bicarbonate buffer, pH 9.6, and left overnight in a humid chamber at 4°C.
2) The ELISA plates will be washed three times with PBS.
3) Then 50µl of the FMDV antigen diluted in blocking buffer will be added to each well of the ELISA plates. (Blocking buffer: 0.05% [w/v] Tween 20, 10% [v/v] NBS, 5% [v/v] normal rabbit serum.) The plates will be covered and placed on an orbital shaker at 37°C for 1 hour, with continuous shaking.
4) After washing three times with PBS, 40µl of blocking buffer will be added to each well, followed by 10µl of test sera (or control sera), giving an initial serum dilution of 1/5.
5) Immediately 50µl of guinea-pig anti-FMDV antiserum diluted in blocking buffer is added, giving a final serum dilution of 1/10.
6) The plates are covered and incubated on an orbital shaker at 37°C for 1 hour.
7) After washing three times with PBS, 50µl of anti-guinea-pig Immunoglobulin conjugate (preblocked by incubation for 1 hour at room temperature with an equal volume of NBS) diluted in blocking buffer will be added. The plates will be covered and incubated for 1 hour at 37°C on an orbital shaker.
8) After washing three times with PBS, 50µl of substrate solution, containing 0.05% H2O2 plus orthophenylene diamine or a suitable alternative chromogen, will be added to each well.
9) The reaction will be stopped after 10 minutes by the addition of 50µl of 1 M sulphuric acid. The plates will be read at 492 nm on a spectrophotometer linked to a computer.

10) *Controls:* On each plate two wells will be used for conjugate control (no guinea-pig serum), four wells each for strong and weak positive sera, two wells for negative sera, and four wells for 0% competition (no test sera).
11) *Interpretation of the results:* A percentage of inhibition will be calculated for each well, either manually or using a suitable computer programme (100 – [optical density of each test or control value/mean optical density of the 0% competition] × 100%), representing the competition between the test sera and the guinea-pig anti-FMDV antisera for the FMDV antigen on the ELISA plate.
12) A spectrophotometer will be used to measure the optical density (OD) at 450 nm. The test will be considered validated if the mean value of the negative control OD was greater than 0.8 and the positive control serum will be expected to give $\geq$90% inhibition at 1/10 dilution and >50% inhibition at the second dilution (1/30). This assay will be used selected neutralizing anti-FMDV monoclonal antibodies specific for FMDV serotypes to measure specific antibodies against the particular serotypes. The test will be performed according to the manufacturer's manual. Results will be calculated by the percentage inhibition of positive control and by test sera: %inhibition = 100- (serum OD / reference OD*) x 100; *Reference OD = mean OD of four wells processed with the Negative Control. And the interpretation will be done as shown below:

    Positive when producing an inhibition >70% at the 1/10 dilution.

    Negative when producing an inhibition < 70% at the 1/10 dilution.

    Reference OD = mean OD of four wells will be processed with negative control.

Appendix 6: Consent Form

Good morning/good afternoon! My name is Tekalegn Desta Tucho. Currently I am a post graduate student of Addis Ababa University College of Veterinary Medicine and Agriculture, department veterinary Microbiology, Immunology and Public Health. Now I am conducting research on **"Outbreak Investigation of Foot and Mouth Diseasein Cattle in Central and Southern Ethiopia".** I will be grateful if you take part in this research. The study's objectives are serotyping of FOOT AND MOUTH DISEASEV, Isolation of the virus, and outbreak investigation of the disease, estimate the seroprevalence of FOOT AND MOUTH DISEASEin cattle population and assess associated risk factors introducing the FOOT AND MOUTH DISEASEto the cattle population of the study area through questionnaire preparation. To accomplish the goal of this study, I need your assistance. You will not receive any payment or special treatment for taking part in the study. Your name won't be listed on the question sheet, making it impossible for anyone to identify you for any reason. All information you provide will be kept private and unavailable to third parties. You are free to decline participation at any point after you have begun, or you can choose not to participate at all. Your open responses to these questions, however, will aid in my understanding of the FOOT AND MOUTH DISEASEV causes of economic significance and animal deaths in dairy farm and in pastoral communities. Ultimately, this understanding will aid in the creation and implementation of appropriate policies and initiatives to help the country overcome related issues.

Respondent statement: I have understood the above statements:

• Yes (Agree to participate)

• No (Not agree to participate)

Informed consent: I have read this form or it has been read to me in the language I understand all conditions stated above. Therefore, I am willing to participate in this study.

Name of respondent: _____

Signature: _____

Date: _____

Appendix 7: Questionnaire survey and Blood sample collection format.

GENERAL INFORMATION

i. Name of the owner ----------

ii. Physical Address District ---------- (PA) ---------- Village ----------

---

iii. Date of interview ----------

iv. Geographical coordination: lat ---------- long ---------- Alt ----------

2. SOCIO-DEMOGRAPHIC CHARACTERSICS

i. Gender Male ☐ Female ☐

ii. Age (year) : <30 ☐ 30 – 39 ☐ 40 – 49 ☐ 50 - 59 ☐

>60 ☐

iii. Marital status: Married ☐ Single ☐

iv. Occupation: Household ☐ Employee ☐ other ☐

v. Level of education: illiterate ☐ Elementary ☐ High School ☐

degree ☐

3. KNOWLEGDE AND ATTITUDE RELATED TO DISEASE RISK FACTORS.

3.1 KNOWLEGDE RELATED TO DISEASE RISK FACTORS

3.1.1. Have you heard about FMD before? YES ☐ NO. ☐

If yes what symptoms of the disease did you see?

Excessive salivation? ☐

Ulcers on tongue and gums? ☐

Lesions on hooves and lameness? ☐

Lesions on Udder/teats? ☐

Others ☐

3.1.2. How long you have been seen this disease symptom?

Before six months? ☐

Less than six months? ☐

3.1.3 From where do you think the animal get the disease?

From the recently animals come to the area/from market? ☐

From herds or animals having contact with the neighboring animals? ☐

From wild life contact? ☐

3.2 ATTITUDE RELATED TO DISEASE RISK FACTORS.

3.2.1 Do you vaccinate your animals against the FMDV?

3.2 .2. What would you do if you suspected that your herd had FMD?

Report to vet. Professionals ☐

Treating the animals ☐

Do nothing ☐

Sell cattle to village butcher ☐

Isolate the animals (quarantined)

3.2 .3. What would you do to prevent or control FMD?

Treatment ☐

Vaccination ☐

Others/traditional treatment ☐

Blood sample collecting format.

No.	District	Animal ID	Age	Sex	Body condition	Herd size
1						
2						
3						
4						
5						

Appendix 8: Photo gallery

a) b) c)

The Figure a, b and c above shows the study population, FMD lesions on interdigital space and tongue in Salamago district.

Figure d, e & f above shows the FMD lesions on Cattle in Hawassa, Habas and Halaba kulito.

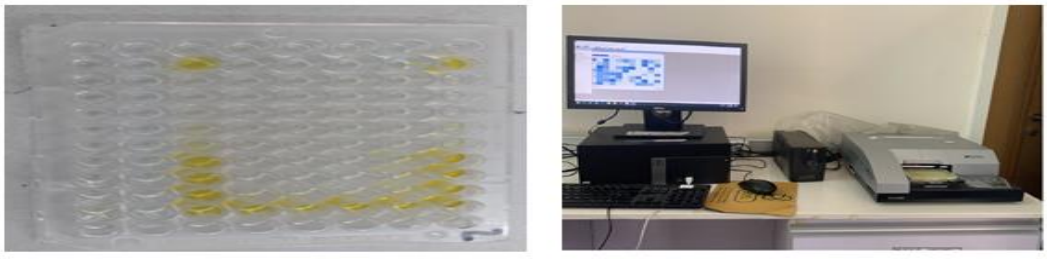
g) h) i)

Figure g, h & i above shows the Bulls foamy salivation and tissue and swab sample collection in Akaki kality site

During the serum preparation from whole blood.

a) b)

Figure a & b above indicates the microplate layout paper preparing, serum sample dispension, and serial Washing in the AHI Viral serology.



c) d)

Figure c and d above shows the microplates with serially diluted samples and during reading FMD serotypes identification by spectrophotometer.

During tissue grinding and prepare hommogenized sample suspension under BSL2 in Viral serology laboratory.

a) b) c)

Figure a, b & c above shows the Forward and Reverse primers, and 3D probe Reagents used and RTPCR master mix preparation.

Appendix 9: Officially signed certificate for laboratory-tested FMD samples by AHI.

1. Serotypes Identification Solid phase ELISA confirmed
2. Serotypes Identification by Antigen Captured ELISA

AHI

PARTIAL TEST RESULT REPORT FORM

Customer name: Tekalegn Desta
Contact: phone : 0922201144, email : desta.tekalegn@gmail.com
Sampling Place: Region: Addis Ababa, District: A/kality Worda 07
Kebele: AI center

Reference No. SR/Ou/462/2024
Test Report No. 462/2024

Date reported: 24-10-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed	Owner
22573-22580	tissue	Bovine	18/10/2024	21/10/2024	23/10/2024 23/10/2024	AI center

Samples type: tissue - Species: Bovine

Samples	Sample Id	Test	Test Result	Method	SOP
1	AI-01	FMDV O	Positive	Capture ELISA	
1	AI-02	FMDV O	Positive	Capture ELISA	
1	AI-03	FMDV O	Positive	Capture ELISA	
1	AI-04	FMDV O	Positive	Capture ELISA	
1	AI-06	FMDV O	Positive	Capture ELISA	
1	AI-07	FMDV O	Positive	Capture ELISA	
1	AI-08	FMDV O	Positive	Capture ELISA	
1	AI_05	FMDV O	Positive	Capture ELISA	
1	AI-01	FMDV A	Negative	Capture ELISA	
1	AI-02	FMDV A	Negative	Capture ELISA	
1	AI-03	FMDV A	Positive	Capture ELISA	
1	AI-04	FMDV A	Positive	Capture ELISA	
1	AI-06	FMDV A	Positive	Capture ELISA	
1	AI-07	FMDV A	Negative	Capture ELISA	
1	AI-08	FMDV A	Negative	Capture ELISA	
1	AI_05	FMDV A	Negative	Capture ELISA	
1	AI-01	FMDV C	Negative	Capture ELISA	
1	AI-02	FMDV C	Negative	Capture ELISA	
1	AI-03	FMDV C	Negative	Capture ELISA	
1	AI-04	FMDV C	Negative	Capture ELISA	
1	AI-06	FMDV C	Negative	Capture ELISA	
1	AI-07	FMDV C	Negative	Capture ELISA	
1	AI-08	FMDV C	Negative	Capture ELISA	
1	AI_05	FMDV C	Negative	Capture ELISA	
1	AI-01	FMDV Asia1	Negative	Capture ELISA	
1	AI-02	FMDV Asia1	Negative	Capture ELISA	
1	AI-03	FMDV Asia1	Negative	Capture ELISA	

NOTE: the results shown on this test report only refers the number of samples tested and the respective customer not for other purpose. AHI is responsible for all the information provided on this report, except for the information provided by the customer.

Effective Date: 2022.07.25	Document Type: FRM	Approved by: Abera Kebede Document Number: AHI-FRM-QEN-025-8A

P.O BOX 04, Sebeta Town, Ethiopia ☎ +251113380694/95/96/97 Fax: +251113380220 Website: http://www.ahi.gov.et/

Verified by:
Dr Demeke Zewdie

Approved by:
Dr.Getnet Abie Mekonnen
Deputy Director General

AHI

PARTIAL TEST RESULT REPORT FORM

Customer name: Takalegn Desta(Dr)
Contact: phone : , email : desta.tekalegn@gmail.com
Sampling Place: Region: Addis Ababa, District: kality worda 10
Kebele: A.A city

Reference No. SR/Ou/464/2024
Test Report No. 464/2024

Date reported: 24-10-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed	Owner
22584-22588	tissue	Bovine	18/10/2024	21/10/2024	23/10/2024 23/10/2024	A.A City

Samples type: tissue - Species: Bovine

Samples	Sample Id	Test	Test Result	Method	SOP
1	k-01	FMDV O	Negative	Capture ELISA	
1	k-02	FMDV O	Negative	Capture ELISA	
1	k-03	FMDV O	Positive	Capture ELISA	
1	k-04	FMDV O	Positive	Capture ELISA	
1	k-05	FMDV O	Positive	Capture ELISA	
1	k-01	FMDV A	Negative	Capture ELISA	

Opinions and Interpretations

Of five tissue samples tested for FMD Ag detection ELISA for different serotypes, three were positive for serotype O and two were negative, and and all other serotypes were not detected.

Verified by:
Dr Demeke Zewde

Approved by:
Dr.Getnet Abie Mekonnen
Deputy Director General

AHI

PARTIAL TEST RESULT REPORT FORM

Customer name: Dr Garuma Desa
Contact: phone : , email :
Sampling Place: Region: SNNP, District: Selamago
Kebele: Omo Hana

Reference No. SR/Su/21/2024
Test Report No. 21/2024

Date reported: 11-03-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed
1595-1626	SERUM	Bovine	22/12/2023	12/01/2024	24/01/2024 01/02/2024

List of Positive Samples: FMD virus: Ab detection / NSP-ELISA

SR No	Lab Code	Sample's Id	Result	Location
1	1595	525	Positive	Omo Hana
2	1596	526	Positive	Omo Hana
3	1598	528	Positive	Omo Hana

NOTE: the results shown on this test report only refers the number of samples tested and the respective customer not for other purpose. AHI is responsible for all the information provided on this report, except for the information provided by the customer.

		Approved by: Abera Kabede
Effective Date: 2022.07.25	Document Type: FRM	Document Number: AHI-FRM-GEN-025-8A

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ATTACHMENT

List of Positive Samples: FMD virus: Ab detection / NSP-ELISA

SR No	Lab Code	Sample's Id	Result	Location
4	1606	536	Positive	Omo Hana
5	1607	537	Positive	Omo Hana
6	1609	539	Positive	Omo Hana
7	1611	541	Positive	Omo Hana
8	1612	542	Positive	Omo Hana

Opinions and Interpretations
Out of 32 sera samples,11 were positive and 21 negative for 3ABC FMD virus antibody.

Verified by:
Dr Demeke Zewde

Approved by:
Dr.Getnet Abie Mekonnen
Deputy Director General

Demeke Sibhatu
(BSC,MVSc)

AHI

TEST RESULT REPORT FORM

Customer name: Takalegn Desta(Dr)
Contact: phone : , email : desta.tekalegn@gmail.com
Sampling Place: Region: Sidama, District: Hawassa city
Kebele: 03
Sampling type: Purposive

Reference No. SR/Ou/448/2024
Test Report No. 448/2024
Date reported: 24-12-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed	Owner
21211-21211	tissue	Bovine	17/10/2024	17/10/2024	08/11/2024 11/11/2024	03

Samples type: tissue - Species: Bovine

Samples	Sample Id	Test	Test Result	Method	SOP
1	10	FMDV O	Negative	Capture ELISA	
1	10	FMDV A	Negative	Capture ELISA	
1	10	FMDV C	Negative	Capture ELISA	
1	10	FMDV Asia1	Negative	Capture ELISA	
1	10	FMDV SAT1	Negative	Capture ELISA	
1	10	FMDV SAT2	Negative	Capture ELISA	
1	10	FMDV SAT3	Not tested	Capture ELISA	

*Accredited Test (AHI is accredited for tests marked *)

NOTE: the results shown on this test report only refers the number of samples tested and the respective customer not for other purpose. AHI is responsible for all the information provided on this report, except for the information provided by the customer.

		Approved by: Abera Kabede
Effective Date: 2022.07.25	Document Type: FRM	Document Number: AHI-FRM-GEN-025-8A

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Verified by:
Dr Demeke Zewde

Dr Redeat Belayneh

Approved by:

Dr.Getnet Abie Mekonnen
Deputy Director General

AHI

PARTIAL TEST RESULT REPORT FORM

Customer name: Tekalegn Desta
Contact: phone : , email :
Sampling Place: Region: Oromiya, District: Gatarse
Kebele: Guje

Reference No. SR/Ou/463/2024
Test Report No. 463/2024

Date reported: 24-10-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed	Owner
22581-22583	tissue	Bovine	18/01/2024	21/10/2024	23/10/2024	Guje
					23/10/2024	

Samples type: tissue - Species: Bovine

Samples	Sample Id	Test	Test Result	Method	SOP
1	01	FMDV O	Positive	Capture ELISA	
1	02	FMDV O	Positive	Capture ELISA	
1	03	FMDV O	Positive	Capture ELISA	
1	01	FMDV A	Negative	Capture ELISA	
1	02	FMDV A	Negative	Capture ELISA	
1	03	FMDV A	Negative	Capture ELISA	

NOTE: the results shown on this test report only refers the number of samples tested and the respective customer not for other purpose. AHI is responsible for all the information provided on this report, except for the information provided by the customer.

		Approved by: Abera Kabede
Effective Date: 2022.07.25	Document Type: FRM	Document Number: AHI-FRM-GEN-025-8A

PO BOX 04: Sebeta Town, Ethiopia +251113380894/95/96/97 Fax: +251113380220 Website: http://www.ahi.gov.et/

Verified by:
Dr Demeke Zewde

Approved by:

Dr.Getnet Abie Mekonnen
Deputy Director General

AHI

PARTIAL TEST RESULT REPORT FORM

Customer name: Dr Garoma Desa
Contact: phone : , email :
Sampling Place: Region: SNNP, District: Dasenech
Kebele: Kolomagnato
Kebele: Ariquel
Kebele: Delegnemur

Reference No. SR/Su/18/2024
Test Report No. 18/2024

Date reported: 11-03-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed
1337-1490	SERUM	Bovine	25/12/2023	12/01/2024	24/01/2024 01/02/2024

List of Positive Samples: FMD virus: Ab detection / NSP-ELISA

SR No	Lab Code	Sample's Id	Result	Location
1	1337	267	Positive	Kolomagnato
2	1338	268	Positive	Kolomagnato
3	1339	269	Positive	Kolomagnato
4	1341	271	Positive	Kolomagnato
5	1342	272	Positive	Kolomagnato
6	1343	273	Positive	Kolomagnato
7	1344	274	Positive	Kolomagnato
8	1345	275	Positive	Kolomagnato
9	1346	276	Positive	Kolomagnato
10	1347	277	Positive	Kolomagnato
11	1348	278	Positive	Kolomagnato
12	1349	279	Positive	Kolomagnato
13	1350	280	Positive	Kolomagnato
14	1351	281	Positive	Kolomagnato
15	1354	284	Positive	Kolomagnato
16	1356	286	Positive	Kolomagnato
17	1357	287	Positive	Kolomagnato
18	1358	288	Positive	Kolomagnato
19	1359	289	Positive	Kolomagnato
20	1360	290	Positive	Kolomagnato
21	1361	291	Positive	Kolomagnato
22	1362	292	Positive	Kolomagnato
23	1363	293	Positive	Kolomagnato
24	1364	294	Positive	Kolomagnato

Testing Lab	No.	Positive	Negative	Doubtful	Test	Method	SOP
AHI	154	148	6	0	FMD virus: Ab detection	NSP-ELISA	AHI-TM-SRL-009*

*Accredited Test (AHI is accredited for tests marked *)

Opinions and Interpretations

Out of 154 sera samples,148 were positive and six were Negative for 3ABC FMD antibody.

Verified by:
Dr Demeke Zewde

Approved by:
Dr.Getnet Abie Mekonnen
Deputy Director General

AHI

TEST RESULT REPORT FORM

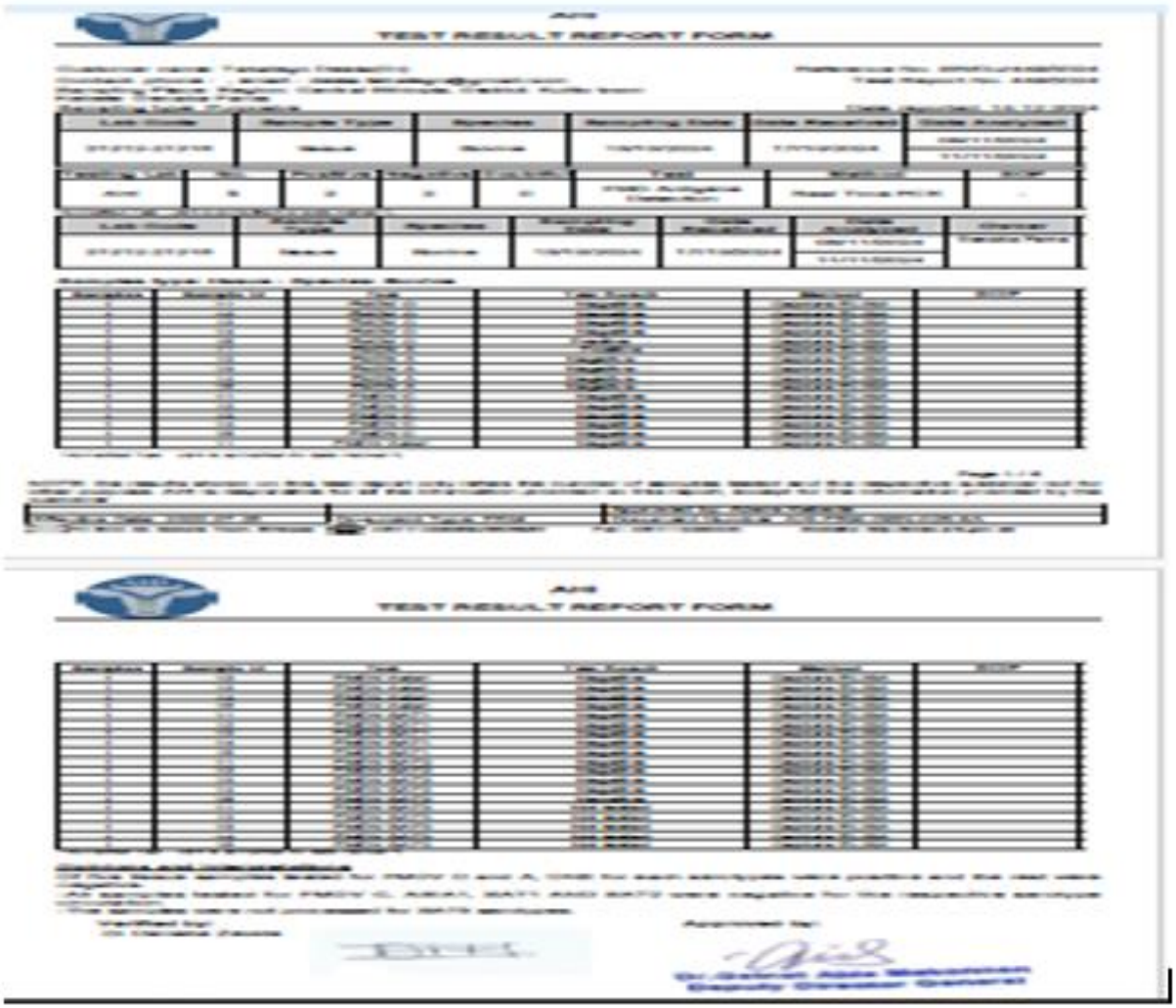
Customer name: Takalegn Desta(Dr)
Contact: phone : , email : desta.tekalegn@gmail.com
Sampling Place: Region: Central Ethiopia, District: Kulito town
Kebele: Denaba Fama
Sampling type: Purposive

Reference No. SR/Ou/449/2024
Test Report No. 449/2024
Date reported: 14-12-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed
21212-21216	tissue	Bovine	13/10/2024	17/10/2024	08/11/2024 11/11/2024

Testing Lab	No.	Positive	Negative	Doubtful	Test	Method	SOP
AHI	5	2	2	0	FMD Antigene Detection	Real Time PCR	-

*Accredited Test (AHI is accredited for tests marked *)



AHI

TEST RESULT REPORT FORM

Customer name: Takalegn Desta(Dr)
Contact: phone : , email : desta.takalegn@gmail.com
Sampling Place: Region: Oromiya, District: Habas
Kebele: 01
Kebele: 02
Sampling type: Purposive

Reference No. SR/Ou/447/2024
Test Report No. 447/2024
Date reported: 24-12-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed	Owner
21202-21208	tissue	Bovine	10/10/2024	17/10/2024	08/11/2024 11/11/2024	01

Samples type: tissue - Species: Bovine

Samples	Sample Id	Test	Test Result	Method	SOP
1	1	FMDV O	Negative	Capture ELISA	
1	2	FMDV O	Negative	Capture ELISA	
1	3	FMDV O	Negative	Capture ELISA	
1	4	FMDV O	Negative	Capture ELISA	
1	5	FMDV O	Negative	Capture ELISA	
1	6	FMDV O	Negative	Capture ELISA	
1	7	FMDV O	Negative	Capture ELISA	
1	1	FMDV A	Negative	Capture ELISA	
1	2	FMDV A	Negative	Capture ELISA	
1	3	FMDV A	Positive	Capture ELISA	
1	4	FMDV A	Positive	Capture ELISA	
1	5	FMDV A	Negative	Capture ELISA	
1	6	FMDV A	Negative	Capture ELISA	
1	7	FMDV A	Negative	Capture ELISA	
1	1	FMDV C	Negative	Capture ELISA	
1	2	FMDV C	Negative	Capture ELISA	
1	3	FMDV C	Negative	Capture ELISA	
1	4	FMDV C	Negative	Capture ELISA	
1	5	FMDV C	Negative	Capture ELISA	
1	6	FMDV C	Negative	Capture ELISA	
1	7	FMDV C	Negative	Capture ELISA	
1	1	FMDV Asia1	Negative	Capture ELISA	
1	2	FMDV Asia1	Negative	Capture ELISA	
1	3	FMDV Asia1	Negative	Capture ELISA	
1	4	FMDV Asia1	Negative	Capture ELISA	
1	5	FMDV Asia1	Negative	Capture ELISA	

*Accredited Test (AHI is accredited for tests marked *)

NOTE: the results shown on this test report only refers the number of samples tested and the respective customer not for other purpose. AHI is responsible for all the information provided on this report, except for the information provided by the customer.

Effective Date: 2022.07.26	Document Type: FRM	Approved by: Abera Kebede Document Number: AHI-FRM-GEN-025-5A

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Verified by:
Dr Demeke Zewde

Approved by:
Dr.Getnet Abie Mekonnen
Deputy Director General

Dr Redeat Belaynen

AHI

PARTIAL TEST RESULT REPORT FORM

Customer name: Dr Garoma Desa
Contact: phone : , email :
Sampling Place: Region: SWNNPR, District: Jinka
Kebele: Napitikoyit
Kebele: lopokor
Kebele: Ayipa
Sampling Place: Region: SNNP, District: Gnangatom
Kebele: Napitikoyit
Kebele: lopokor
Kebele: Ayipa

Reference No. SR/Su/15/2024
Test Report No. 15/2024

Date reported: 11-03-2024

Lab Code	Sample Type	Species	Sampling Date	Date Received	Date Analyzed
1071-1198	SERUM	Bovine	22/12/2023	12/01/2024	24/01/2024
					20/02/2024

List of Positive Samples: FMD virus: Ab detection / NSP-ELISA

SR No	Lab Code	Sample's Id	Result	Location
1	1071	1	Positive	Ayipa
2	1073	3	Positive	Ayipa
3	1074	4	Positive	Ayipa
4	1075	5	Positive	Ayipa
5	1076	6	Positive	Ayipa
6	1077	7	Positive	Ayipa
7	1078	8	Positive	Ayipa
8	1079	9	Positive	Ayipa
9	1080	10	Positive	Ayipa
10	1082	12	Positive	Ayipa
11	1083	13	Positive	Ayipa
12	1084	14	Positive	Ayipa
13	1085	15	Positive	Ayipa
14	1086	16	Positive	Ayipa
15	1087	17	Positive	Ayipa
16	1089	19	Positive	Ayipa
17	1090	20	Positive	Ayipa
18	1091	21	Positive	Ayipa
19	1092	22	Positive	Ayipa
20	1093	23	Positive	Ayipa
21	1097	27	Positive	Ayipa
22	1098	28	Positive	Ayipa

Testing Lab	No.	Positive	Negative	Doubtful	Test	Method	SOP
AHI	128	95	33	0	FMD virus: Ab detection	NSP-ELISA	AHI-TM-SRL-009*

*Accredited Test (AHI is accredited for tests marked *)

Opinions and Interpretations

Out of 128 sera samples,94 were positive and 34 were negative for 3ABC FMD virus antibody.

Verified by:
Dr Demeke Zewde

Approved by:
Dr.Getnet Abie Mekonnen
Deputy Director General

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/02/61/16/2025

Name of Applicant: **Tekalegn Desta** (DVM, MSc student)

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project *Foot and mouth disease outbreak investigation in selected areas of central and southern parts of Ethiopia*

Date of application:	**December, 2023**
Nature of the project:	**Field investigation**
Target animal species:	**Cattle**
Number of animals involved:	**368**
Study area:	**Central and southern part of Ethiopia**

Minutes No. and date of review: **VM/ERC/02/16/024, 23/01/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Tekalegn Desta.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

ፋክስ} ስልክ } ፖ.ሣ.ቁ ቢሾፍቱ፤ ኢትዮጵያ
Fax 251-11-4339933 Tel. +251 114338450 P.o.x. Box}34 Bishoftu, Ethiopia

Takalegn

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